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COLOR CHANGES IN ANIMALS, THEIR SIGNIFICANCE AND ACTIVATION[1]

INTRODUCTORY REMARKS

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ANIMALS are marvelously resourceful in meeting the impact of their surroundings. Even such inert and apparently innocuous creatures as sea-anemones and jellyfishes can often inflict a sting excruciatingly painful to man and deadly to many fishes. Thus these simple inhabitants of the sea protect themselves against insult and kill other creatures for their food. All animals generate electricity in greater or smaller amounts. To the expert physician the beating of the human heart can be read and studied in terms of almost imperceptible electric changes, but the electric eel of South America can deliver a bolt that will knock down a horse or kill a frog. All animals secrete substances which may vary from the hardness of the needle-like fang of a rattlesnake to the fluid venom injected through such a fang. Heat is produced by all animals and light by a goodly number. The luminous creatures of sea and land have been the inspiration of poets from the days of Homer to the present time, and Oriental nations celebrate as national festivals the coming of the firefly. Bodily movements and their attendant locomotion are commonly looked upon as animal responses par excellence. Most such activities are accomplished by

[1] A symposium given at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938.

muscles which give us the lumbering gait of an elephant, the astounding flight of a bird, the beat of an insect's wing so rapid often as to produce an audible note and the unequalled modulations of the human voice. But animal movements are not only muscular. They may be due to the whipping of innumerable, minute, protoplasmic lashes or cilia which like myriads of microscopic oars propel small water creatures through their aqueous environment. Or these movements may be due to the sluggish flowing or snail-like creeping of the living substance of such microscopic animals as the ameba whose semi-fluid body seems to flow over the solid background of its watery abode. Nor are we in our own bodies devoid of such activities, for our white blood-corpuscles migrate within us by the same means that the ameba uses and assemble where they are needed to attack and destroy such invading organisms as the germs of disease.

To this form of motility, a kind of viscous or sarcous streaming, are to be attributed many of the color changes in animals. These changes are commonly carried out by the displacement of the colored contents within certain cells, the chromatophores, located in the skins of those animals that easily change their tints. When myriads of such cells of a given shade and in the integument of a chromatic animal disperse their pigment, they confer on the creature their characteristic tone. When these cells draw in their special coloring matter and concentrate it about their centers, the tint previously shown by the animal disappears. Thus the creature by controlling the movements of its various chromatophoral pigments may change its color over a range that is limited only by the number of kinds of color cells in its skin. Any one who has watched a flounder swimming over a diversified, sandy bottom will have observed the play of colors that pass over its body as cloud shadows pass over a landscape. So regular and progressive are these waves of color as they course over the skin of this fish that it seems almost incredible that they should be due to an orderly repeated

expansion and contraction of a multitude of color cells. Yet such is the truth.

That animals are able to change their tints was known to the ancients, for Aristotle, the father of natural history, described these phenomena in the chameleon and in the devil-fish. His account of these changes is so circumstantial and detailed that we are forced to believe that he must have been acquainted with them at first hand. The means by which such changes are brought about were discovered, however, only a little over a century ago and the term chromatophore as the name for the integumentary agent concerned with such changes was first used in 1819 by the Italian Sangiovanni. Shortly after that time it was discovered that the eyes of chromatic animals were essential to their color changes, for when these organs were effectually covered or the animal otherwise blinded, it ceased to respond to color differences in its surroundings. The involvement of the eyes in color responses led to the assumption that the color cells of animals were under the control of their nerves and this view, well supported by much experimental evidence, maintained itself throughout the last century. Since the eyes and other nervous parts of animals were thus included in their chromatic organization, it followed that this type of system was to be expected only in the higher creatures. It was therefore not surprising to find that color changes with very few exceptions proved to be limited to these animals. The ability to exhibit color changes is found among certain mollusks such as the squids and devil-fishes, among crustaceans as represented by the shrimps, prawns and crabs, and, lastly, among the cold-blooded vertebrates, the fishes, amphibians and lizards. All these animals are highly organized in that they possess well-developed eyes and nervous systems capable of high degrees of efficiency. Nor was there before the end of the last century reason to suppose that more than these parts were necessary for a full understanding of animal color changes.

For many years past it has been known that the injec-

tion of certain drugs and other like materials into the bodies of chromatic animals is often followed by changes of color. In 1898 it was discovered by two Italians that when small amounts of adrenalin were injected into frogs, these animals became very pale in tint. Adrenalin is a product of the adrenal gland, an organ of internal secretion, and its function is that of an activator or hormone, to use a technical designation. Thus an internal secretion in the form of the hormone adrenalin was shown to be a possible means by which color cells could be brought into action. Less than two decades after this, intermedin, the hormone from the intermediate lobe of the pituitary gland of the brain, was seen to have a most profound influence on the chromatophores of many animals. The effect of this hormone was the reverse of that of adrenalin, for while adrenalin made animals pale, intermedin made them dark. As a result of these discoveries and of others of a like kind, it was shown that color cells in animals were controlled not only by nerves but by internal secretions in the form of hormones or, as they are specifically termed nowadays, neurohumors.

But this distinction, important and significant as it seemed to be, is apparently doomed to disappear, for the most recent work on this subject indicates that the nervous control of color cells is in reality accomplished also by secreted substances, substances that are liberated from the ends of the nerves as other activating materials are discharged from glands. If this view proves to be correct, then all instances of chromatophoral activation, nervous as well as hormonal, are dependent upon these substances, some of which, like intermedin, come from distant glands and are carried to the color cells by the blood and others from nerve endings in closest proximity to the reacting color elements themselves. Such is the most recent opinion of the way in which color changes are controlled. By a delicate adjustment of the amount of these activating substances or neurohumors, by a balance of such agents, so to speak, and by their appropriate interplay, any chro-

matic animal may attain to a given tint or succession of tints such as is needed in its daily life.

The conception that the cells of the nervous system and of its appended parts, such as muscles, glands and chromatophores, are linked together not by electric intercommunication as believed of old but by activating substances such as these described in this brief survey is steadily gaining ground. It is the working hypothesis of a host of modern investigators in nerve physiology. That human thought with all its implications, both normal and pathological, as well as so distant an activity as the color changes of animals should rest upon a background of chemical activation is a view that promises rich and important yields in the future investigation of the whole range of nervous phenomena.

It must be evident from what has been said in these introductory remarks that the subject of color changes in animals is one of fascinating complexity. Its investigation may be carried out from many standpoints. The papers which make up this symposium will lead you to see how diversified this attack may be.

CHROMATOPHORES IN RELATION TO GENETIC AND SPECIFIC DISTINCTIONS[1]

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WE as zoologists are familiar with the extraordinary diversity of the characteristics of living things. Our classifications are based largely on structural similarities and diversities. The lesser subdivisions, such as genus, species, variety and Mendelian phenotypes, usually utilize the more readily visible characters. Among the latter are colors and color patterns—as, for example, the distinction between the zebra and the horse. My purpose in this paper is to survey the relative degree to which these taxonomic and genetic differences may be displayed by color patterns, by color cells—the chromatophores, and by pigments. Among these, however, I wish to pay especial attention to the cells. We may inquire as to what extent do chromatophores of different families, genera, species or genetic phenotypes exhibit recognizable distinctions. In accordance with prevailing beliefs we may state that the characteristics of the organism are conditioned by the interaction of all its genes; and moreover most of the cells of the individual are thought to contain this full complement of genes. May we then not expect to find that homologous cells in species A and B or even in genetic phenotypes A and B differ recognizably on account of their differing gene content? That this is to some extent true has

[1] Read at a symposium on "Color Changes in Animals, Their Significance and Activation" at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938. When presented it was illustrated in part by lantern slides of color photomicrographs (Kodachrome) which can not be reproduced here. I am greatly indebted (especially for the photography) to my assistant, Miss Marian Hedenburg. Many of the photographs were taken at the New York Aquarium by authorization of the acting director, Dr. C. M. Breder, Jr., and with the help of Dr. C. W. Coates and Dr. R. F. Nigrelli, to all of whom I wish to express my thanks. Expenses incurred were defrayed by grant from the Denison Fund for Biological Research at Wesleyan University.

long been recognized by the comparative histologists and by the embryologists who take advantage of recognizable cell differences for demarking the boundaries of interspecific tissue transplants. A not infrequent statement made by students who work with individual living cells, either in the organisms or in tissue cultures, is that they can by long familiarity recognize the cells of different species, even though it is difficult to formulate these distinctions in words. Protozoologists distinguish many different species of *Amoeba* by criteria hard to describe, as they must be applied to a "formless" animal. It is my desire to call attention to a few observations that have been made bearing on the question of the possibly recognizable specificity of pigment cells of fishes.

We may first consider the phenotypic distinctions due to single gene differences. Perhaps the most notable and clear-cut cases are those described by Gordon in a series of papers dealing with *Platypoecilus maculatus* (Gordon, 1931). The character "spotted" is produced by very easily recognizable cells, the macromelanophores, and these appear only when gene "Sp" is inherited. Similarly, character "stippling" is caused by an abundance of micromelanophores which follow gene "St." When gene "Sp" is united in an interspecific hybridization with the genetic complex of the swordtail fish, *Xiphophorus helleri,* there occurs an extraordinary overdevelopment of macromelanophores forming distinctive melanotic tumors.

In *Oryzias* (*Haplocheilus*) *latipes,* first studied by Aida (1921) and later in greater histological detail by Goodrich (1927), a normally pigmented melanophore follows gene B, while the recessive allelomorph b produces a faintly pigmented cell. The difference between these two has been shown (Goodrich, 1933) to be apparently due to lack of sufficient chromogen in the light-colored cell, although sufficient oxydase is present. In this fish, however, the situation is complicated by the fact that a third allelomorph B′ produces a situation such that approximately equal numbers of dark and light cells are produced. This

probably indicates that the control of these cell differences is at least in part due to factors in embryonic development other than the gene content of individual cells, such for example as an embryonic induction.

A very interesting interspecific cross between the mackerel, *Scomber scombrus,* and the killifish, *Fundulus heteroclitus,* was first made by H. H. Newman in 1918. A more detailed study of the history of chromatophores in this cross which confirms and adds to his observations is now being made by Dr. Alice M. Russell, who has kindly given me permission to refer to her unpublished work. The embryos resulting from this hybridization do not proceed to maturity. In embryonic stages, however, some individuals show, apparently unchanged, the distinctive green chromatophores of the mackerel and others the red brown chromatophores of the killifish, or both may be present in the same embryo. Here then the results indicate that the chromatophores follow the shufflings of the hereditary genic complexes. In this same cross, according to Dr. Russell's observations, it is more difficult to be certain of the relations of melanophores of the two parental types.

Single gene differences may control the presence or absence of cell types. This is illustrated by the total absence of melanophores in an albino mutant contrasted with the normal, as in the paradise fish, *Macropodus opercularis,* described by Goodrich and Smith (1937). There are many types of fancy tropical fish which probably only await genetic analysis to reveal cases of cell types controlled by single gene differences.

A second phase of our inquiry is concerned with the question as to how extended a distribution of practically identical cell types exists among related varieties, species or genera. This is a field in which at present there is insufficient material for forming the basis of a confident generalization. A preliminary exploration, however, indicates different degrees of constancy of cell types in different families and genera of fish. Gordon's studies on *Platypoecilus,* which revealed the existence of two types

of melanophores, as noted above, are now being extended (Gordon, 1937) in an investigation of the closely related *Xiphophorus helleri,* a fish which has an overlapping geographic range with *Platypoecilus maculatus* and which hybridizes with it. Here he finds the same type of micromelanophores as in *Platypoecilus* (Fig. 3, *Platypoecilus*; Fig. 4, *Xiphophorus*) and certain similar color patterns. On the other hand, as mentioned above the macromelanophores can not be introduced into *Xiphophorus* without disastrous results. Our observations also indicate that a distinctive erythrophore having red granular periphery and yellowish center is found in both of these related species. The genetics of a beautiful xanthophore which we have observed in a variety of *Xiphophorus helleri* known to breeders as the blood-red swordtail is as yet unknown. Varieties of *Mollienisia sphenops,* known to fanciers as the "blue mollie" and the "liberty mollie," have strikingly similar melanophores. Taking now an example of distinct species in the same family, Figs. 1 and 2 show the similarity of the heavy melanophores of *Macropodus opercularis* and of *Betta splendens,* species of the family Osphronemidae. Two species of the family Haemulidae, or the grunts, recently examined at the New York Aquarium have similar delicate melanophores (Figs. 5, 6). I have recently been interested in the family Labridae, or the wrasses, which includes many brilliant coral reef fish. Species have been studied at the Tortugas laboratory in the Gulf of Mexico and at the laboratory of the University of Hawaii, and although these were so widely separated geographically they show some degree of resemblance of the melanophores and especially of the iridocytes (Figs. 7, 8).

The similarities may seem more real when they are compared with existing diversities of cell types. The family Cyprinidae, or the carps, is very large and wide-spread, and genera show great diversity as is illustrated by photographs of cells from *Rasbora trilineata* (Fig. 9) and *Brachydanio rerio* (Fig. 10). Types from widely sepa-

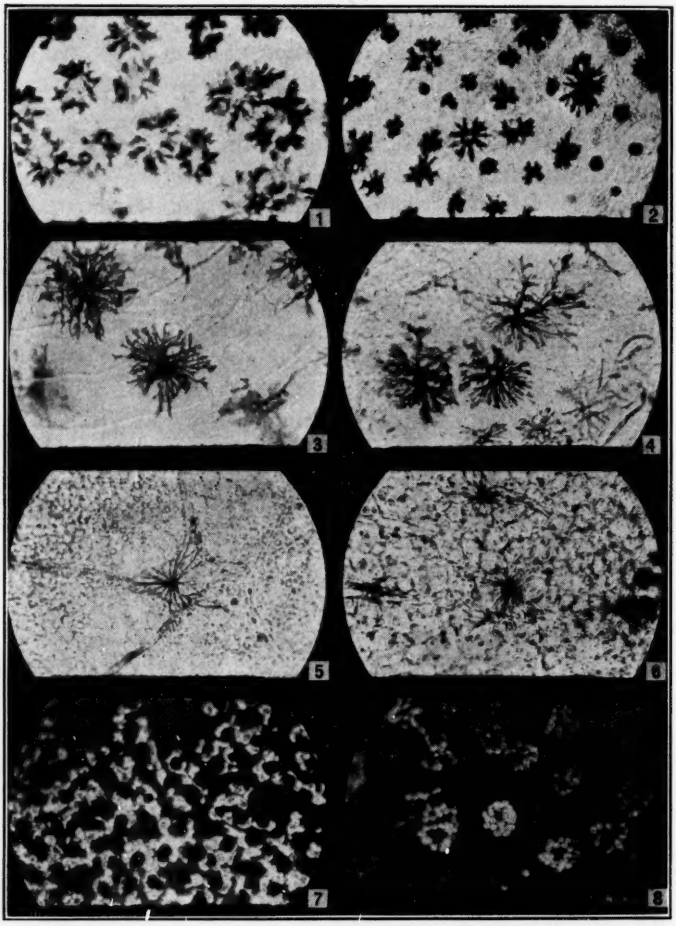

PLATE I

Comparison of melanophores of closely related species or genera. Photomicrographs. Figs. 1–6, ×163, Figs. 7–8, ×88 magnification. Family Osphronemidae. Fig. 1. *Macropodus opercularis* Linnaeus (Paradise fish). Fig. 2. *Betta splendens* Regan (Siamese fighting fish). Family Poeciliidae. Fig. 3. *Platypoecilus maculatus* Guenther ("Platy"). Fig. 4. *Xiphophorus helleri* Meek (swordtail). Family Pomadasidae. Fig. 5. *Haemulon macrostomum* Guenther (gray grunt). Fig. 6. *Haemulon flavolineatum* Desmarest (yellow grunt). Family Labridae. Photographs to show iridocytes, melanophores contracted. Fig. 7. Dark stripe on *Halichoeres bivittatus* Bloch (slippery dick). Fig. 8. Blue zone on *Thalassoma bifasciatum* Bloch (bluehead).

rated families are shown in Plate II. These include in addition to the carps the following: *Fundulus heteroclitus* (Fig. 11) from family Cyprinodontidae; *Lophopsetta maculata* (Fig. 12), family Pleuronectidae; *Holocentrus ascencionis* (Fig. 13), family Holocentridae; *Pseudoscarus guacamaia* (Fig. 14), family Scaridae; *Julis eydouxi* (Fig. 15), family Labridae; *Centrophistes striatus* (Fig. 16), family Serranidae.

The preceding discussion may have seemed to imply that I have held that the nature of cells is directly and wholly determined by their own gene content. But of course the possible influence of cell environment must not be forgotten. Indeed such an effect is clearly indicated by sex demorphism in such types as the "guppy," *Lebistes reticulatus,* and *Xiphophorus helleri,* and confirmed by cases of sex reversal and experiments with sex hormones (*cf.* Berkowitz, 1938). In this latter case there is the clear indication of the suppression of the male color pattern by feeding immature fish with the female sex hormone. These circumstances indicate that the differentiation of certain chromatophores, or even their presence or absence, may be dependent on chemicals circulating in their environment. The distinction between the control of a cell's structure directly by its own nuclear constitution and an effect produced on it by its environment is the same as that which has been drawn in recent literature in the field of physiological genetics and referred to as the difference between genic and endocrinal regulation of tissues, but what I would prefer to refer to as the distinction between immediate and remote genic control. Splendid examples of this are known from the studies on bird plumage (*cf.* reviews by Danforth, 1932, and by Domm, Juhn and Gustavson, 1932). For our immediate inquiry, however, we prefer to try to eliminate variables and so are concerned with cells which do not vary between the sexes or seem to be otherwise differentially affected by their environment (as in Oryzias cited above).

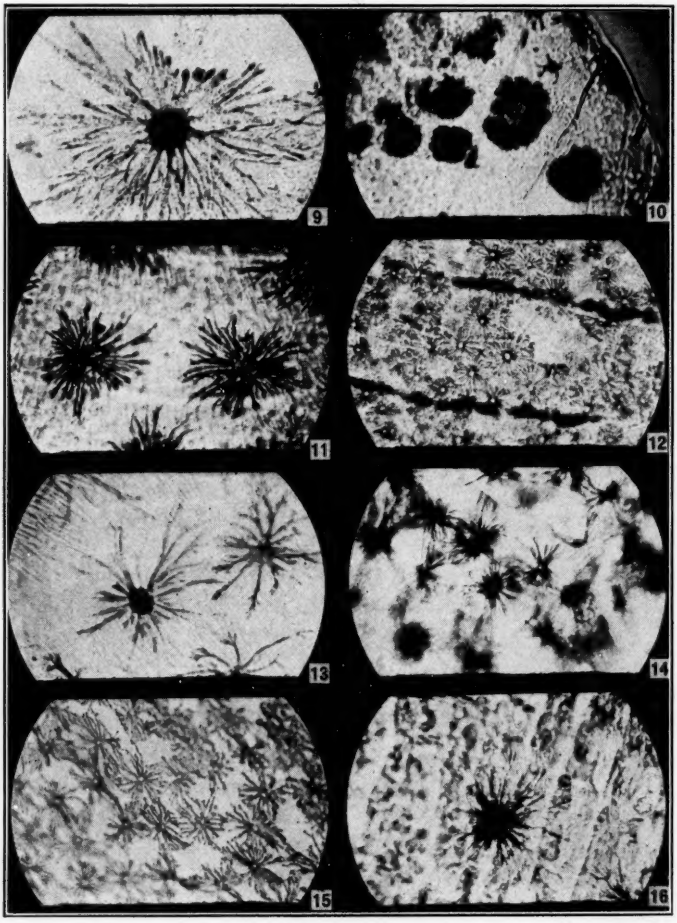

PLATE II

Diverse types of melanophores. Photomicrographs all ×163. Family Cyprinidae. Fig. 9. *Rasbora trilineata.* Fig. 10. *Brachydanio rerio* Hamilton-Buchanan (zebra fish). Practically no pseudopodia appear on these melanophores. Family Cyprinodontidae. Fig. 11. *Fundulus heteroclitus* Linnaeus. Family Pleuronectidae. Fig. 12. *Lophopsetta maculata* Mitchell (sundial). Family Holocentridae. Fig. 13. *Holocentrus ascensionis* Arbeck (squirrel fish). Family Scaridae. Fig. 14. *Pseudoscarus guacamaia* (rainbow parrot fish). Family Labridae. Fig. 15. *Juliseydouxi* Cuvier and Valenciennes. Family Serranidae. Fig. 16. *Centropristes striatus* Linnaeus (sea bass).

The photographs of chromatophores that we have shown indicate, I believe, that these cells exhibit in many cases a specific individuality, although the distinctions may be evasive and hard to analyze. Also in some cases chromatophores of related species and even of related genera show a considerable degree of similarity. The most obvious similarities are matters of the size of the cell and the form, length, branching and number of its pseudopodia. In all cases, however, caution is necessary, as the appearance of the individual cell varies greatly according to the degree of concentration or distribution of its contained pigment.

Turning now to the coloring material we may state that pigment appears to vary less frequently than the cell types. The structural formula of melanin is not yet known. It does not appear that there are many melanins. The coloring materials of xanthophores and erythrophores probably are carotenoids, and it has been suggested that there may be about 100 different carotenoids. Quite possibly the estimate may be increased by biochemists, but the order of magnitude is not likely to approach that of the approximate number of species of fish—about 15,000. Therefore we may assume that frequently the same pigment will appear in many different species of fish.

From the preceding discussion we may tentatively conclude that while a chemical cytoplasmic cell component, the pigment, may be wide-spread among many species, the cell type has a much more limited distribution, which may be less than or somewhat more extensive than the species. The most obvious and probably most real differences between species that are based on colors are caused by variations in the spatial arrangements of the color-producing units, *i.e.*, the cells. Effects are produced in two ways, by microscopic and by macroscopic groupings or patterns. The former may produce visually different colors. For example, brilliant blues are formed most frequently by the association of melanophores and iridocytes. Erythrophores or xanthophores may be present. In greens the

proportion of xanthophores is increased. No blue or green pigment is usually present. I have observed that the brilliant bluish color of the head region of a coral reef fish, *Thalassoma duperrey*, from Hawaii, is produced by a background of melanophores, iridocytes, and erythrophores upon which are superimposed little islands of melanophores and glittering iridocytes apparently floating on the transparent epidermis. The following table shows counts of chromatophores per square millimeter made from certain coral reef fish of the family Labridae.

TABLE I

CHROMATOPHORES PER SQUARE MILLIMETER

	Stripes		
	Black	*Blue*	*Green*
Thalassoma bifasciatum (Tortugas)			
Melanophores	718	346	462
E plus X*	0–550	566	0–190
Iridocytes	3,000	3,000	3,000
Thalassoma duperrey (Hawaii)			
		Yellow	*Green*
Melanophores		271	596
Xanthophores		1,057	527
Iridocytes		3,000	3,300

* Erythrophores plus Xanthophores.

The macroscopic effects are the obvious groupings which produce stripes, bands, spots, and in fact almost any conceivable configuration. The macroscopic groupings and possibly the microscopic arrangements are distinctive of each species. It is then in this realm of the larger units of organization that we find the maximum expression of the possibilities of biological color variation. The chemicals seem least variable, the cells more variable, and the configurations of color patterns most variable of all. This of course does not seem strange, because biologists, aided by the philosophers of science, have distinguished between various degrees of organization. We are familiar with the categories of electronic, atomic, molecular and biological levels of organization. And within the biological we distinguish between the cellular and the multicellular. The last, having available greater possi-

bilities of spatial arrangement, is logically the most variable and therefore the best level of organization for expression of biological specific differences.

Passing over this last philosophical digression, the chief points which I wish to emphasize are the following two: (1) that cells may have an individuality which like that of the whole organism is an expression of its genic complex, though probably more affected by some genes than by others, and (2) that the limits of distribution of a cell type may not always be coextensive with the species. Its distribution may be less or sometimes even greater than the species.[2]

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[2] Diagrams which illustrate excellently the idea that taxonomic characteristics may or may not vary with the species and quite independently of each other will be found in papers by Alfred C. Kinsey (Fig. 8) and G. G. Simpson (Fig. 7) presented in a recent symposium before the American Society of Naturalists.

THE PITUITARY CONTROL OF CHROMATOPHORES IN THE DOGFISH*

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THE color changes of elasmobranch fishes were not discovered until about six years ago. In spite of the numerous contributions during this short interval, there exists much diversity of opinion concerning the mechanisms controlling elasmobranch metachrosis. This paper will summarize the various present opinions of chromatic control, and in addition will present some original data which indicate the possibility of a basically uniform plan of chromatic behavior for this group.

The ability to adapt to white or black backgrounds is not shared by all elasmobranchs thus far investigated. These fishes may be roughly divided in this respect into two groups: one showing background responses (macroscopic or microscopic), which are evident within a few hours or a day or longer (*Mustelus canis,* Parker and Porter, 1934; *Raia branchiura, Raia maculata, Rhina squatina, Scyllium canicula* and *Scyllium catulus,* Hogben, 1936; *Squalus acanthias,* Waring, 1938; *Trigon pastinaca, Raia undulata,* Vilter, 1937) and another showing no background adaptation or, at best, limited responses visible only after many days or weeks of exposure (*Raia clavata, Raia batis,* Schaefer, 1921, *Torpedo marmorata,* Veil and May, 1938; *Urolophus testaceus, Trygonorrhena fasciata,* Griffiths, 1936). The first three species of the latter group are more or less persistently dark in tint. No diurnal rhythm of chromatic behavior has been reported in elasmobranchs.

When the pituitary gland or only the neuro-intermediate lobe of this gland is removed, all elasmobranchs thus far

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investigated become completely pale, due to extreme melanophore contraction, and remain in this condition in environments which elicit darkening in unoperated animals (*Mustelus canis,* Lundstrom and Bard, 1932; *Squalus acanthias,* Lewis and Butcher, 1936; *Scyllium canicula, Raia brachiura, Raia maculata, Rhina squatina, Scyllium catulus, Raia clavata, Raia microcelata,* Hogben, 1936; *Torpedo marmorata,* Veil and May, 1937; *Trigon pastinaca, Raia undulata,* Vilter, 1937; *Raja erinacea,* this paper). However, such hypophysectomized specimens may be darkened temporarily by the implantation of the neuro-intermediate lobe or by the injection of an appropriate extract from this lobe of the hypophysis. From this and other evidence to be pointed out later, all investigators are agreed that the dark phase of elasmobranch color change is due to the production and circulation of the chromatophorotropic hormone (intermedin, "B" hormone, melanophore hormone, etc.) of the neuro-intermediate lobe of the pituitary.

Although investigators are in complete agreement concerning the mechanism controlling the dark phase, no less than four theories have been proposed to account for the induction of the pale or light phase:

(1) That blanching or melanophore contraction is due to the production and circulation of a new hypophyseal hormone—the "W" or melanophore contracting hormone of the anterior lobe of the elasmobranch pituitary (the dualistic hormone theory, Hogben, 1936, Waring, 1938, pertinent to all elasmobranchs).

(2*a*) That paling is due to the direct intervention of melanophore contracting nerves, carrying visual impulses, initiated by a white background, to the melanophores by means of a lipoidal neurohumor as a chemical mediator ("The Nervous Theory," Parker, 1935; pertinent chiefly to *Mustelus canis* and also to *Squalus acanthias*).

(2*b*) That pallor is due to a tonic action of the nervous system allowed expression when the influence of the pituitary is removed or diminished. As in 2*a,* a neurohumor

(sympathin) is postulated as a chemical mediator for the induction of melanophore contraction. (Vilter, 1937; pertinent probably to all elasmobranchs).

(3) That pallor is brought about by the diminution or cessation of intermedin secretion during adaptive responses to a white background ("Unihormonal Theory," Lundstrom and Bard, 1932; Parker, 1936–1938; pertinent to *Mustelus canis, Raja erinacea, Squalus acanthias.*

The remainder of this paper will be devoted to an examination of these theories with respect to the original data obtained from experiments on the smooth dogfish, *Mustelus canis.* Such a procedure may be criticized on the general ground that various elasmobranchs differ in their mechanisms of control and, therefore, that a discussion of the group based on results of only one member is unwarranted. However, in *Mustelus,* evidence pertaining to all three main theories has been obtained, and consequently this species has proven to be for the present the most desirable experimental animal.

The responses[1] of *Mustelus* to backgrounds and to certain operative measures are shown in Table I. Although some of these responses have been described fully by Lundstrom and Bard (1932) and Parker (1936–1938), a quantitative tabulation is presented in this paper not merely for the purpose of verification, but chiefly to indicate the extent of variation. Three significant responses may be noted:

(1) That the neuro-intermediate lobe is the only portion of the pituitary, the removal of which results in complete melanophore contraction.

(2) That the removal of only the anterior lobe results in the loss of the white background response.

(3) That the melanophores do not respond directly to light. Concerning the third point, it is true that blinded elasmobranchs are darker when illuminated than when in

[1] The diameter of the pigment mass within the melanophores (melanophore diameters) was determined on living fish by means of a calibrated ocular micrometer. 10–25 melanophore diameters were recorded for each fish, and the average taken.

TABLE I

MELANOPHORE RESPONSES IN THE PUPS OF MUSTELUS CANIS
(After one day adaptation to background)

Condition of animals	White background			Black background			Darkness		
	Number of animals	M.D.	S.D.	Number of animals	M.D.	S.D.	Number of animals	M.D.	S.D.
Normal	5	74	16	10	191	18	4	141	3
Optic nerves cut	4	178	13	4	170	7	4	165	3
Complete hypophysectomy* plus Optic nerves cut	3	32	0.3	2	32	0.5	4	30	2
Complete hypophysectomy.	4	29.2	1.0	4	29.2	1.0	5	29.2	1
Neuro-intermediate lobectomy	6	32.4	7.0	5	29.2	3.0	5	27.5	2
Anterior lobectomy	4	186	13	5	196	8	2	181	12
Ventral lobectomy	4	90	8	6	144	24	2	152	32
Sacculus vasculosus extirpated	6	94	36	2	175	13	4	141	37
Neuro-intermediate lobectomy plus sacculus extirpated	2	29.2	0.3	1	37	...	1	32	..

M.D. = Melanophore diameter in micra.
S.D. = Standard deviation in micra.
* = Extirpation of anterior, neuro-intermediate, and ventral lobes, and also sacculus vasculosus.

darkness. The justification of describing this response as a direct effect of light on melanophores (Hogben, 1936; Waring, 1938) is questionable, since it does not occur in blinded, hypophysectomized specimens.

We may now consider the first of the four proposed mechanisms for the induction of the pale phase—the "W" hormone theory.

(1) EXTIRPATION OF THE ANTERIOR LOBE

The removal of the anterior lobe results in the loss of the white background response, the animals remaining persistently dark in tone in spite of long sojourns on a white background. First demonstrated by Hogben (1936) for *Scyllium canicula, Scyllium catulus* and *Raia brachiura,* this effect has been confirmed by Waring (1938) for *Squalus acanthias,* and in this paper for *Mustelus canis* and *Raja erinacea.* That the loss of the white background response results specifically from the extirpation of the anterior lobe, thereby depriving the animals of a "W" hormone, is doubtful, as reference to Table II indicates. This table shows that the same response may be effected by

TABLE II

EFFECT OF SUPERFICIAL BRAIN LESIONS MADE IN WHITE ADAPTED PUPS (MUSTELUS CANIS) ON MELANOPHORE RESPONSES

(5 animals per group)

Type of lesion	Melanophore diameters in micra				
	On white background			Placed on black on 4th day	Returned to white on 5th day
	Days after lesion				
	1st	3rd	4th	5th	6th
Transverse 1–2 mm between optic chiasma and anterior lobe	243	234	243	243	243
Transverse 1–2 mm anterior to optic chiasma	65	65	49	243	50
Transverse 3–5 mm caudad to pituitary	49	65	65	243	41
2 longitudinal lesions parallel with long axis of anterior lobe (3–5 mm)	40	73	97	243	110
"Denervated" pituitary[1]	240	240	240	240	240
"Implanted" pituitary[2]	240	240	240	...	...
Anterior and neuro-intermediate lobes separated, but not extirpated	162	180	200	180	190

[1] Tongue of anterior lobe dissected free from hypothalamus, then hypophysis completely freed from brain. Hypophysis attached now to brain case by ventral lobe.

[2] Same as above with the exception that anterior and neuro-intermediate lobes also cut free from ventral lobe.

superficial brain lesions made so as to interrupt the probable path of nerve supply (supraoptico-hypophyseal tract) to the intermediate lobe, even when the anterior lobe remains intact. In view of these experimental findings, therefore, the first line of direct evidence favoring the "W" hormone theory loses much of the significance generally attached to results following the surgical removal of an endocrine gland. The results of anterior lobectomy seem explicable as readily on the ground that the mechanism of inhibiting intermediate lobe secretion is interfered with as on the basis that a "W" hormone is no longer available to the melanophores. Certainly, in view of the results listed in Table II, the latter view can not be unequivocally maintained.[2]

(2) REPLACEMENT THERAPY

If the anterior lobe produces a "W" hormone, implants of the anterior lobe or injections of extracts of it should

[2] It may be argued that brain lesions affect B and W hormone production differently. My experiments, of course, do not rule out the existence of a W hormone. They do show, however, that for the present the effect of anterior lobectomy must be regarded as non-specific.

restore the white background response in anterior lobectomized animals. Waring (1938) has found that such measures are wholly ineffective. By other tests (isolated dogfish skin, test on *Xenopus*) Waring (1936) and Hogben (1936) have not been able to demonstrate the presence, in the elasmobranch anterior lobe, of a substance which contracts melanophores.

(3) Tests for a "W" Hormone in the Blood of Pale Animals

If a "W" hormone is responsible for pallor, its presence in the blood of pale animals should be detectable. Parker (1936–1938) injected blood of pale animals (*Raja erinacea, Squalus acanthias, Mustelus canis*) into dark specimens, without effect on the melanophores of the latter. My own attempts[3] to detect the "W" hormone in the blood of animals possessing intact anterior lobes but lacking neurointermediate lobes (such specimens should contain "W" hormone but no "neutralizing" B hormone) have also failed. In these experiments the test blood was injected intramuscularly into animals possessing intact intermediate lobes but lacking anterior lobes.[4]

(4) Differential Tolerance to "B" Hormone

Waring (1938) has shown that *Scyllium canicula,* when injected with a given dose of "B" hormone, will remain dark longer when the anterior lobe is absent than when it is present. This differential tolerance to equal doses of "B" is interpreted as being due to the presence of an antago-

[3] Subcutaneous injections of blood (0.2–0.5 cc) from normal pale, neurointermediate lobectomized and completely hypophysectomized animals into dark hosts (normal or anterior lobectomized) always produced pale spots. Sea-water likewise produced pale areas; hence the response is non-specific. None of these effects were produced on intra-muscular injections.

[4] It might be argued that these experiments do not rule out the presence of a "W" hormone in the blood since, by injection of pale blood into dark animals, any "W" present would be neutralized by the antagonistic "B" substance present in the host. Unless insufficient amounts of blood were injected, it is difficult to see how these experiments could otherwise be carried out.

nistic and neutralizing "W" hormone, hence animals with anterior lobes (and without neuro-intermediate lobes) do not remain dark as long as animals deprived of both lobes. Such a differential tolerance may be explained readily on other grounds: (1) that the anterior lobe may produce a substance inactivating "B" and yet not be a melanophore contracting hormone in itself, and (2), which is more likely, that the change in metabolism following anterior lobectomy in many animals may apply also to elasmobranchs, in which case the differential tolerance would be simply a differential metabolism of the injected "B" hormone.

Viewing all the evidence for the dualistic theory, it seems that a "W" hormone has not been demonstrated beyond reasonable doubt. The weakest evidence, peculiarly, results from the direct attack. The circumstantial evidence (differential tolerance curves) is stronger, but explicable on other grounds. The experiments herein reported do not rule out the existence of a "W" hormone. They do indicate, however, that the "W" hormone theory should not, for the present, be accepted until better direct evidence is obtained.

The nervous theory was first formulated by Parker (1935–1938), who discovered (Parker and Porter, 1934) that chisel cuts made transverse to the rays of the pectoral fin or that faradic stimulation of these rays produce pale areas. As a general mechanism of blanching in elasmobranchs, the nervous theory is not applicable, and Parker has limited it to *Mustelus* and possibly to *Squalus*, although not to all individuals of the latter species. Most of the elasmobranchs studied show no nervous control of melanophores. Section of spinal, trigeminal and facial nerves in *Scyllium* sp. has no effect on melanophores (Young, 1933). Similarly, negative effects have been obtained in *Scyllium canicula* by pectoral chisel cuts (Waring, 1936) or by section of spinal nerves (Wykes, 1936); in *Raja erinacea* by pectoral chisel cuts (Parker, 1937); in *Rhina squatina* and *Scyllium catulus* by electrical stimulation of spinal nerves and of the haemal canal (Wykes, 1936); in *Raia maculata*

by section of spinal nerves (Wykes, 1936); and in *Raia brachyura* by section of spinal nerves, or by faradic stimulation of spinal nerves or of the haemal canal (Wykes, 1936). In *Squalus acanthias,* electrical stimulation of several rays of the pectoral fin is ineffective in producing pallor (Parker, 1936) but chisel cuts across the rays sometimes produce pale areas (Parker, 1936; Waring, 1938). In *Torpedo,* Vilter (1937) induced pallor by faradic stimulation of the spinal cord. It appears therefore that more evidence has been accumulated for a nerve control in *Mustelus* than in any other elasmobranch, for in addition to cutting and stimulating experiments, Parker (1935) has been able to extract from pale fins a lipoidal neurohumor which he believes to be the chemical mediator to the melanophores.

It must be emphasized that a chisel cut in the pectoral fin of *Mustelus* does more than transect nerves. That the circulation distal to the cut may be interfered with is a criticism that has been repeatedly voiced. Parker has maintained, however, that collateral circulation is evident in the area affected by the cut, but whether this collateral supply is sufficiently normal in its rate of flow and in its total supply is as yet unknown. My studies of the arterial pattern of the pectoral fin indicate that in the area of the fin where cuts are commonly made, it is an anatomical impossibility to avoid severing several radial arteries in denervating by means of a chisel cut. If it could be shown that when nerves are surgically isolated and severed, the area thus denervated would pale, much of the doubt of the interpretation of pale fin bands would be dispelled.

By a dorsal incision over the pectoral girdle of *Mustelus,* the brachial plexus has been exposed and each nerve entering the plexus has been cut, but subsequent pallor in the fin has not been observed. That such an operation denervates the entire fin is seen from resulting immobility of the fin in swimming movements. Even when spinal nerves 1–13 were severed, no paling was observed. On the contrary, background responses may be induced in the denervated

fin. Similarly, section of the superficial ophthalmic nerve, on one side or both, produces no paling of the head region. Spinal nerves 21–26 were cut, either consecutively or alternately, but pale areas were not produced. If a chisel cut is made on a denervated pectoral fin, a pale band will appear within 10–30 minutes. If the fin of a dark fish is ligated, the entire fin will pale within 10–30 minutes. But, if a chisel cut is made at the same time the fin is ligated, no pale band will appear. Instead, the entire fin pales uniformly. When the ligature is removed, the fin will darken within 5–10 minutes, but the cut area will remain as a pale band. If a denervated fin is ligated, it will pale within 10–30 minutes.

From these results, it is evident that ligating a fin[5] and making a chisel cut produce the same response in exactly the same time. It is also evident that no melanophore contracting nerves are contained in the brachial plexus at the region of operation. Finally, it appears that if melanophore nerves exist in the pectoral fin of *Mustelus*, they must enter it at some point distal to the region at which the plexus enters the fin. In view of these results[6] showing both the importance of the blood supply and the non-existence of melanophore nerves in the brachial plexus at the point of operation, the nervous control of the pale phase in *Mustelus* can not be accepted wholly until the central connections of such melanophore contracting nerves are found or until histological examination reveals an innervation of the melanophores of this dogfish.

The thesis that melanophores are under a tonic nervous control meets with the same general objection—such nerves must be demonstrated first. An observation by

[5] It is possible that ligation of a fin stimulates the nerves by pressure, and hence the melanophores contract. The observation that section of the plexus close to the point where the ligature was made did not produce pallor can not be cited against such a possibility, inasmuch as the melanophore nerves could enter the fin without being contained in the spinal nerves, or if contained in the plexus, at some point distal to entrance of the plexus.

[6] The nerve-cutting experiments involving the brachial plexus were performed on pups, immature and mature specimens. Ligation experiments performed on pups.

Wykes (1936) that pallor is produced uniformly in innervated as in denervated areas following hypophysectomy tends to discredit the tonicity theory. However, the argument that no melanophore nerves are present in the spinal nerves which were cut by Wykes is justifiable, as in the experiments cited above on severing the brachial plexus. But it must be reaffirmed that all attempts to demonstrate the presence of melanophore contracting nerves by surgical measures have failed, and that until this is successfully accomplished, the nervous theory can not be wholly accepted.

The unihormonal theory remains for discussion. Parker (1936–1938) found that defibrinated blood of dark but not pale animals produced melanophore expansion when injected into pale specimens of *Raja erinacea, Mustelus canis* and *Squalus acanthias.* Waring (1938) has also found that more "B" is present in the blood of *Squalus acanthias* during black adaptation than during white adaptation. My own results, although they are still in a preliminary state, are in accord.

Thus the unihormonal theory is the only one in which all the pertinent evidence is in favor of it, and against which there are at present no serious objections. Furthermore, it is the only plan of control which not only applies to those species for which it has been advocated, but which may also function as a basically uniform plan throughout the group. If concentrating nerves are present in *Mustelus,* it is conceivable that pallor is due to the combined action of both nerves and a diminution of intermediate lobe secretion, either of which could produce this effect alone. In any event, it is difficult to evaluate at present the relative significance of nerve control since, on the one hand, normal paling can be accounted for in terms of the pituitary alone, and, on the other, paling can not occur when the pituitary gland is incapacitated by denervation of other measures.

Summary

Of the various theories of control for the color changes in elasmobranchs, the unihormonal theory is at present the

most satisfactory. This mechanism of control was intimated by Lundstrom and Bard (1932) the pioneer investigators in this field, and first clearly expressed and supported by Parker (1936–1938).

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QUANTITATIVE EFFECTS OF VISUAL STIMULI UPON PIGMENTATION*

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IT is well known by this time, I trust, that the color changes of such animals as possess this capacity are caused in two quite distinct ways. On the one hand, we have relatively rapid changes, sometimes manifested within a few seconds' time, which involve no change in the amount of pigment, but merely the rearrangement of pigment already present. On the other hand, and less familiar, are the more gradual but more enduring changes, which result from the persistent action of adequate stimuli and which involve an actual increase or decrease in the amount of pigment in the animal's tissues. These types of color change have been distinguished, not altogether appropriately, as "physiological" and "morphological," respectively.

It is the former to which by far the greater amount of attention has been devoted in the past. It is the latter with which I shall occupy myself to-day. Owing to the limits of time I shall restrict myself to the pigmental changes of vertebrates.

We are not here concerned with the direct effects of light upon pigmentation, such as we have all experienced in the tanning of our own skins by the sunlight. The production or elimination of pigment to which I refer is brought about indirectly as a response to the background, and is mediated through the eyes and the nervous system.

So far as I know, the cytologist Flemming was the first to point out (1882) that the shade of the background could

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be influential in modifying the quantity of pigment in the skin of an animal. Flemming's observations were made upon larval salamanders.

But Babák (1912, 1913), using the same material, seems to have been the first to experiment with any thoroughness upon the effects of background on pigment formation, and to undertake quantitative comparisons of the resulting changes.[1] Babák counted several times, at intervals, the number of chromatophores in certain regions of the skin of living specimens which were kept on white and black backgrounds. He concludes: "There is thus no doubt that the nervous system, under the influence of the light relations of the environment, controls not only the movements and the mass of the pigment in the chromatophores, but also influences the division or multiplication of the chromatophores." Continued "expansion" of chromatophores, he believed, so altered the nutritive condition of these cells that there results not only an increase in their pigment but also a more rapid multiplication. Continued "contraction," on the other hand, produces reverse effects, though Babák's results indicated little, if any, loss of chromatophores which had once been formed.

Passing reference may be made to the claims of Kammerer (1913), who reported not only that young salamanders responded by pigmental changes to different backgrounds, but that these changes were inherited. Even the former of these contentions was disputed by Herbst (1919, 1924, 1927) at least for post-larval salamanders, though the experiments of von Frisch (1911) tended to support Kammerer's claims.[2]

Kuntz (1916) contributed a valuable paper on "The histological basis of adaptive shades and colors in the flounder *Paralichthys albiguttus*." He reports a reduction of approximately 30 per cent. in the number of melanophores in the superficial layers of the dermis when

[1] Franz (1910) made some brief observations of this sort on young plaice.

[2] *l.c.*, as to effects on the first generation, but with no reference to inheritance.

specimens which had been kept for some time on white were compared with freshly caught specimens or ones from a black background. A graph which Kuntz presents shows both a decrease in fishes transferred to white backgrounds and an increase when such specimens were returned to black. The maximum effect, he found, was attained in 11 days. Moreover, complete adaptation to white probably involved an increase in the number of guanophores as well as a loss of melanophores.

The massive contribution of Murisier (1920–1921) must likewise be given prominance in any discussion of this subject. This investigator, working on young trout, found that white and black backgrounds strongly influenced the quantity of melanin produced, without appreciably influencing the growth of the fish. For one experiment he reports that the mean number of melanophores counted in the dorsal fins of the white-adapted fishes was 295, that for the black-adapted fishes being 680. Corresponding figures for the anal fins were 4 and 67. Murisier believed, however, that whereas sojourn on black leads to a great increase in the number of melanophores, sojourn on white merely causes an arrest of the further production of these cells, not a loss of those previously present. This is not in agreement with the findings of most others who have experimented on fishes.

Like Kuntz, Murisier was led to believe that stimuli which favored a decrease in melanin production favored an increase in the production of the white substance guanin.[3]

Blinding, he found, favored a great increase in melanin production, at least in fishes kept in the light. This has been the experience of several investigators,[4] including the present writer.

[3] Eva Meyer (1931) likewise contends that a continued sojourn on white leads to an actual increase in the number and size of iridocytes (guanophores). Sumner and Wells (1933) found evidence for an increase in the amount of guanin.

[4] Mayerhofer (1909) for pike, Babák (1912) and Fischel (1920) for Amphibia.

Of importance is the parallel which Murisier was one of the first to stress between the effect of retinal stimulation in bringing about the "contracted" condition of melanophores, and its effect in retarding the multiplication of these cells and upon the formation of pigment.

Himmer (1923) set out to test Babák's contention that morphological color change is a consequence of prolonged physiological color change. He performed the interesting experiment of subjecting larval salamanders to dilute solutions of cocaine, a substance which he found to produce a continued state of contraction of the melanin masses. Thus he aimed to eliminate the immediate effect of visual stimuli in the matter.

Himmer reports a resulting pallor, similar to, but smaller in degree, than that produced by life on a white background. Such a result plainly needs confirmation.

More recently, this same theoretical question has been discussed in the light of the hormonal interpretation of chromatophore action, which we owe so largely to the work of Parker and his collaborators.[5]

Giersberg (1934) attributes the melanin dispersion and subsequent melanin increase in blinded goldfish to the effect of intermedin from the pituitary. The expanded condition of the color cells, he believes, is the cause of the production of new pigment. "The morphological color change is hence to a certain degree a function of the physiological."

Giersberg reports that he injected a young brown goldfish for a period of a year and a half with adrenalin, and that the fish, in consequence, lost its yellow and black pigments. He suggests that life on a white background may likewise favor the production of adrenalin. We may remark even more emphatically in the present case that this experiment with adrenalin calls for confirmation.

Somewhat later, Odiorne (1937) has expressed the alternative view that physiological and morphological color changes are the parallel results of a common (hor-

[5] *Cf.* Parker, 1936. A later bibliography will be found in Parker, 1938.

monal) cause, rather than that the latter is a direct effect of the former.

To return to our chronological review, we must include certain other writers who have recognized the quantitative effect of visual and other nervous stimuli upon pigment formation.

Kurz (1920) exposed the eggs and larvae of *Pleuronectes* and *Esox* to white light, total darkness and to light of five different colors. No effects were observed in the case of the pike, after three months of exposure to these conditions. For the flounders, on the other hand, Kurz reported certain changes in the further development of pigments cells once produced, though not upon their origin.

The work of Kudô (1922) is sometimes seriously cited in discussions of this subject, but his entire report is so unconvincing, and his claims so incredible, that I can not attribute any importance to them. His thesis is that the darkening of a fish, however called forth and apparently of however brief duration, results from an actual increase in the amount of melanin present. Even "Tötung durch Schläge auf den Kopf" produces an increase in this substance! Pictures (*not photographs*) of test-tubes containing various amounts of dark precipitates are offered as evidence for these effects.

The findings of Vilter (1931) upon melanin production in axolotls should be mentioned at this point, though they deserve no such drastic criticism as do those of Kudô. They can hardly be regarded as convincing, however, owing to the small number of animals employed (one each on black and white) and to his very meager account of the methods employed.

Hewer (1927, 1931) working upon English flatfishes, found that an increase in epidermal melanophores occurred as a result of exposing his fishes for two or three weeks to a black background. The numbers both of erythrophores and iridiophores likewise were found to be influenced by the background, the former undergoing an

increase on orange and a decrease on white; the latter undergoing an increase on white.

I shall next report some of the results obtained by myself and associates during the past few years upon quantitative changes in the pigmentation of fishes.

In *Lebistes* (Sumner and Wells, 1933), there was a rather rapid loss of melanin when fishes of "black" history were transferred to a white background. After a few days, it required but low magnification to reveal the presence of numerous degenerating melanophores in the surface of the skin, while in sections large rounded masses of melanin were seen to be passing to the exterior through the epidermis. Returning such bleached fishes to black containers resulted, in a few days, in the appearance of numerous small pigment masses in the dermis, which increased in number and size for some weeks. These changes were not confined to the skin, however, for wide differences were found in the peritoneum and other regions between fishes of black and white history.

Transfers of the guppies back and forth between the two types of background showed that the same individual could alternately acquire and lose chromatophores. Photographs taken for the purpose did not reveal any tendency for newly formed pigment masses to occupy the same positions as those which had recently been eliminated.

Even the most prolonged exposure to a given background failed to render the resulting state of the chromatophores permanent. Fishes reared from birth in black or white containers, even when the light was continued night and day, and throughout their entire lives[6] responded fairly promptly to a reversal of backgrounds, though not so promptly as ones which had been kept for only a month upon a given background, after attainment of maturity.[7]

Our experiments were not quantitative, in the sense that

[6] Even some which represented a third generation reared under these conditions.

[7] These experiments relate chiefly to "physiological" color changes, but the same statements would doubtless apply to "morphological" ones.

an attempt was made at this time to count chromatophores or to measure melanin. But without any such determinations, the differences in question were sufficiently striking.

Odiorne (1933, 1936, 1937) experimented extensively in this field at about the same time that our La Jolla experiments were conducted. He employed fishes of a number of species, chiefly members of the genus *Fundulus*. Like several of his predecessors, Odiorne determined, in some cases, the extent of background influence in terms of the density of chromatophore distribution per unit area of skin. It is of interest that while the loss of melanin upon white backgrounds was everywhere manifest, the occurrence of an increase upon black was far less evident.

The studies of Sumner and Fox (1933, 1935, 1935a) relate to quantitative changes in yellow pigment of the xanthophyll series. Fishes of several species (*Fundulus parvipinnis, Gillichthys mirabilis, Girella nigricans*) were employed in these studies, the xanthophyll being extracted with customary solvents and the resulting solutions assayed colorimetrically. While all these fishes respond in an imitative fashion to yellow backgrounds, as well as to black, white and gray ones, it is of interest that out of the three, *Girella* alone was found to manifest any differences in the actual amount of yellow pigment which it yielded. It is interesting, too, that the amount of xanthophyll present (though not the visible appearance of the fish) appeared to depend upon the shade rather than upon the color (spectral value) of the background. Thus, the greatest amount of xanthophyll was extracted from the almost inky black fishes which had been kept on black, the least from fishes kept on white, while intermediate and nearly equal amounts were yielded by specimens kept on a rather rich yellow and a neutral gray of medium shade. It should be added that *Girella,* in all circumstances, was found to lose xanthophyll under laboratory conditions.[8] The relations stated above indicate, therefore, relative rates of loss.

[8] This statement does not apply, however, to *Fundulus parvipinnis.*

In our studies, no attempt was made to count xanthophores, nor were any microscopic studies made. Abramowitz (1935), however, examined and counted xanthophores in the caudal fin of *Fundulus majalis,* before and after keeping the fishes on black, white, yellow and blue backgrounds. After periods ranging from twelve days to six weeks, increases were noted on yellow and black, decreases on blue and white.

Thus far, the quantitative differences which I have discussed have related entirely to the responses of the fishes, not to the stimuli which called these forth. The backgrounds have been characterized as "black," "white," "gray," etc., without reference to their exact degree of reflectivity nor to the intensity of the incident light. Several years ago the writer and one of his collaborators undertook to determine what quantitative relations might exist between various visual stimuli and the amount of melanin which could be extracted from fishes which had been subjected to these.

To measure these visual stimuli, at least in relative terms, was, of course, fairly simple. To measure the amounts of melanin, even approximately, was not so simple. However, after many months of experimenting, we developed a method which seemed reasonably adequate, and this was applied to a rather considerable series of fishes (Sumner and Doudoroff, 1937).

The fishes (gobies) were kept in glass aquaria, painted on the outside with black, white and two shades of gray. Sets of aquaria thus painted were exposed to two widely different illuminations, these last being in the ratio of approximately 40:1.

After the fishes had been submitted, night and day, to these conditions for nearly three months, an assay of the melanin was undertaken. It was found that the intensity of the illumination played a probable though a minor part in determining the amount of melanin produced (or retained), but that the chief factor in influencing this amount was the shade of the background. Of chief interest was

the fact that the series of melanin values, at least those from the more brightly lighted set of fishes, assumed a logarithmic rather than a linear arrangement when plotted against the albedos of the backgrounds (Figure 1). The

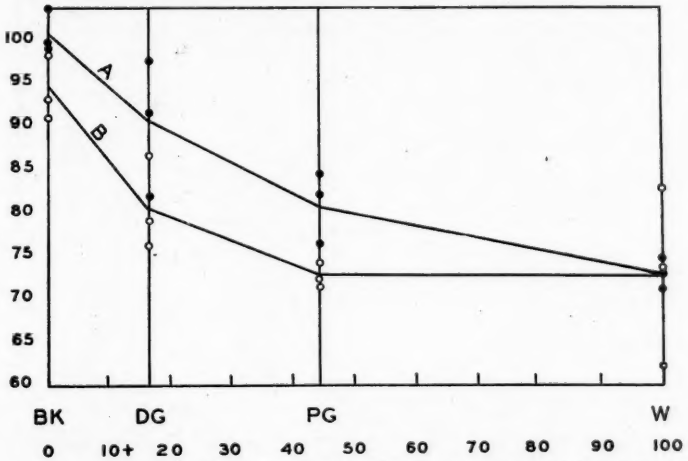

FIG. 1. Curves showing the mean relative melanin values of four lots of fishes (*Gillichthys*) from each of two cabinets. Solid circles represent those from cabinet "A"; open circles those from cabinet "B." Abscissas represent albedos, counting the albedo of the white bowls as 100 per cent. Ordinates represent relative melanin values, taken as percentages of mean value of "black" fishes, regarded as 100. (Modified from Sumner and Doudoroff, 1937).

significance of this last fact will be discussed after I have recorded some confirmatory evidence.

Our experiments with *Gambusia,* more recently reported (Sumner and Doudoroff, 1938), warrant but a brief mention here, owing to the lack of sufficient precision in our determinations. As a result, doubtless, of unperceived variations in our procedure, considerable differences were found between series of fishes which should have given identical results. It deserves reporting, however, that the graphs for the background experiment show a trend similar to that which we encountered in our experiment with gobies, *i.e.,* a more nearly logarithmic than linear arrangement of the values (Fig. 2). On the other hand, no sig-

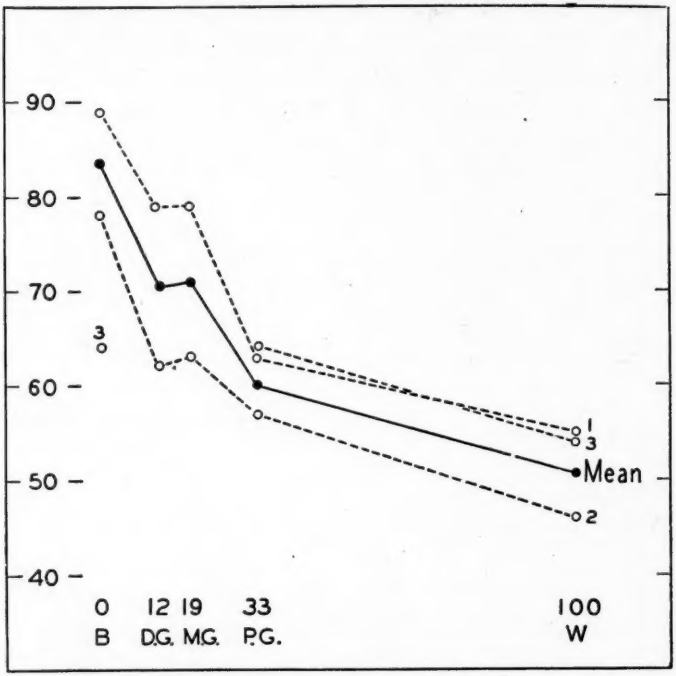

FIG. 2. Computed melanin content of fishes (*Gambusia*), exposed for two months (+) to backgrounds of various albedos. Abscissas represent albedos, counting white as unity; ordinates the melanin content, expressed in milligrams per 100 grams of original weight of fishes. The "mean" curve is based upon the means for series 1 and 2 (From Sumner and Doudoroff, 1938).

nificant differences were detected as a result of the widely different illuminations employed.

It became evident to us that any further effort to assay the melanin of fishes should be deferred until we had overcome certain difficulties of technique. Pending such action, we started a new experiment, this time on *Lebistes*, with a view to counting the melanophores in given unit-areas of the skin.

These fishes were kept in sufficient numbers in bowls of five shades (white, black and three grays) and exposed to light of an intensity of about thirty foot-candles. After

rather more than two months, they were subjected to adrenalin, the effect of which is to bring about the aggregated condition of the melanin and thus to facilitate the counting of the melanophores. The fishes were next fixed, decalcified and cleared in cedar-oil.

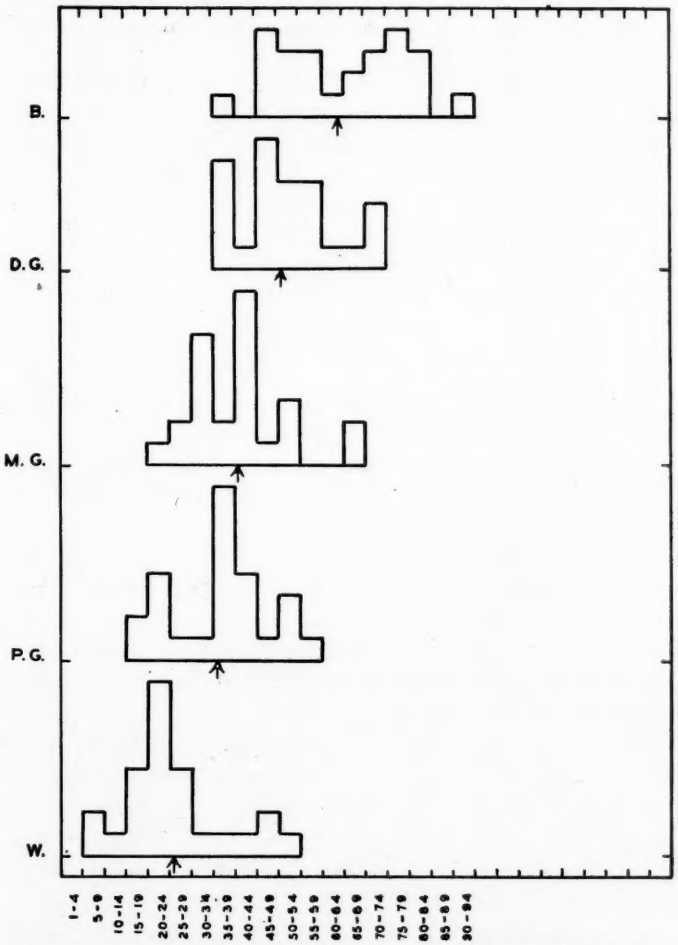

FIG. 3. Frequency distributions of number of melanophores in a given area of skin in premaxillary region of *Lebistes*. Twenty-five individuals from each of five backgrounds: white, pale gray, medium gray, dark gray and black.

Evaluation of results from this experiment is still under way. Using a suitable micrometer grating[9] in the ocular, I have already counted the melanophores in two regions of the skin of twenty-five fishes from each of the five series. Despite high individual variability, the mean differences among these five series appear to be quite significant. (Figs. 3, 4, 5). Most important of all is the defi-

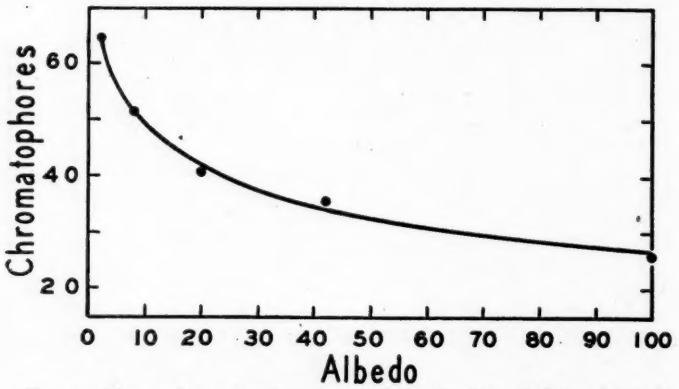

FIG. 4. Mean values of melanophores of the five lots of fishes shown in Fig. 3, plotted against the albedos of the backgrounds, white being regarded as unity.

nitely logarithmic arrangement of the mean values for chromatophore number when plotted against the albedos of the background.

From these facts and others which I cited earlier, it would seem that increments of melanin production vary inversely as the logarithms of the increments of reflectivity of the background.[10] The experimental psychologist has long been familiar with the fact that for some of our senses, at least, and within certain limits, our increments of perception are proportional to the logarithms of the increments of the stimulus (the "Weber-Fechner law"). I believe that our analogy here is more than a

[9] I had employed this method in a preliminary experiment upon *Fundulus parvipinnis* in 1931 (results not published).

[10] Or *vice versa*, the *decrements* of each.

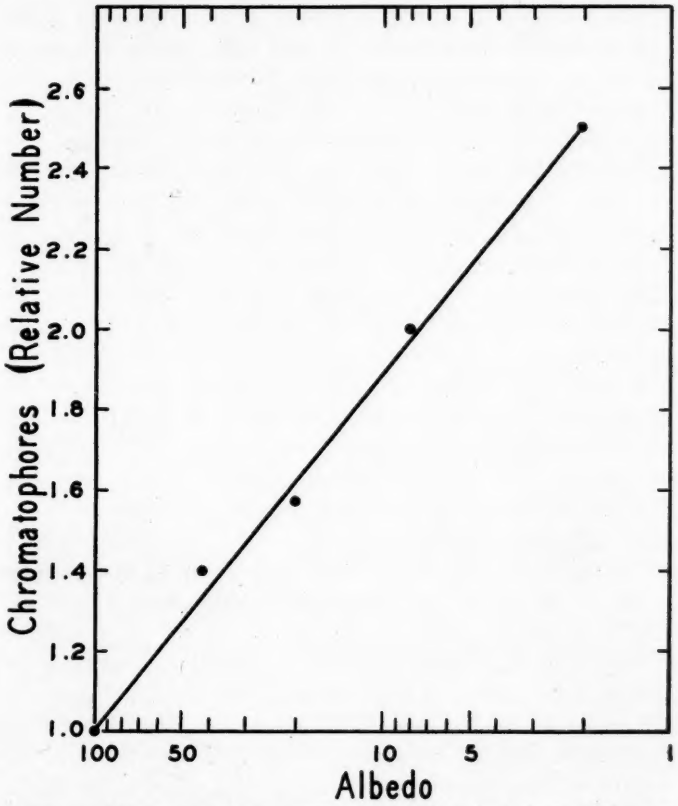

FIG. 5. Mean values shown in Fig. 4, plotted against the logarithms of the albedos. (The albedo of "black" has here been regarded as 2 per cent.)

superficial one. I shall refer again to this case in my address before the Zoologists to-night. (In press, *Scientific Monthly*).

A few words in conclusion regarding this matter of backgrounds. I have spoken glibly of a fish's responding to the albedo of the background, as if a background of a given albedo provided an optic stimulus of constant value. This, of course, is far from the case. The albedo of a surface denotes the proportion of incident light which that surface reflects. The absolute amount of reflected light

varies enormously with the source of illumination. And yet we, in our perceptions, and the fish, in its pigmental responses, appear to recognize the albedo at its true value, largely irrespective of the intensity of the incident light. Both of us, in some way, make allowance for this last.

In 1911, I expressed the view that it was the ratio between the light received from overhead and that reflected by surrounding and underlying surfaces which was chiefly influential in determining the shade assumed by the fish.[11] This view, as it happened, had been expressed in closely similar language by Keeble and Gamble (1904) for certain crustacea.

It may be, therefore, that the upper and lower halves of the field of vision influence the animal in an opposite sense. That the retina of a fish is in some way physiologically polarized is an obvious thought. Attempts to test this hypothesis have been made by several workers. By covering the eyes of *Fundulus parvipinnis* with false corneas of celloidin, variously divided into clear and darkened areas, I obtained some evidence for this view (Sumner, 1933).[12]

Butcher and Adelmann (1937), Vilter (1937) and Butcher (1938) have reported experimental results which point to the same conclusion from rotating the eyeballs of fishes on their axes through an angle of 180°. My own efforts to perform this experiment had been unsuccessful, owing to the drastic procedure involved. However, it seems far from certain that the fish's response to the light relations within its field of vision is regulated entirely by any such

[11] My earlier discussions referred to physiological color changes only. An experimental demonstration of the above proposition was made by Sumner and Keys (1929), and these findings were confirmed by Pearson (1930) for young *Ameiurus melas*. Brown (1936), using more exact quantitative methods, reached the same conclusion for the fish *Ericymba buccata*, except for very low illuminations.

[12] von Frisch (1911) had attempted a similar experiment by applying an opaque paste to the eyes. Butcher and Adelmann (1937) and Butcher (1938) have reported extensive confirmatory results from experiments of this type.

simple polarity of the visual mechanism. It is not true, of course, of our own perception of differences of shade.

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THE RESPONSES OF MELANOPHORES IN ISOLATED FISH SCALES

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THERE are among the vertebrates the following types of effector organs: muscles, glands, cilia, chromatophores and retinal pigment cells. Of these the chromatophore is the only one not found in all groups. Its distribution is limited to the reptiles, amphibians and fishes. Among the chromatophores themselves many different kinds are known, distinguished from each other by the pigment they carry. Of all these perhaps the easiest to study is the fish scale melanophore. This is because the scale is easily removed from the fish without injury to the melanophores, and the scales being translucent the melanophores are readily visualized under the microscope.

The obvious response of the melanophore to stimulation is a change in the distribution of its pigment granules. In Fig. 1, taken from a paper by Spaeth (1913), are shown three different stages of melanin aggregation. The first (a) is conveniently known as the concentrated, contracted or punctate state, the second (b) as the stellate state and the third (c) as the dispersed or expanded state. From time to time various attempts have been made to describe the shift from one state to another. These range all the way from simple visual estimation of the amount of pigment migration to methods for measuring the length of the melanophore process with a micrometer scale. Recently (Smith, 1936) I have succeeded in following these movements with a photoelectric cell by measuring the amount of light transmitted through the scale, which in turn is determined by the state of pigment distribution of

[1] Read at a symposium on "Color Changes in Animals, Their Significance and Activation" at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938.

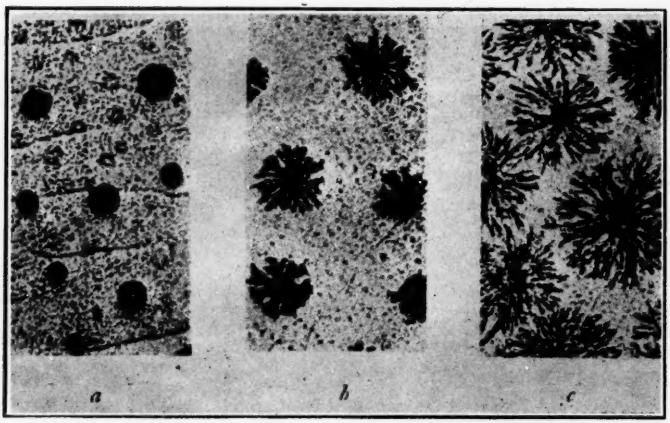

FIG. 1. Isolated scale melanophores of *Fundulus heteroclitus*. *a*, Concentrated or punctate state; *b*, stellate state; *c*, dispersed state. R. A. Spaeth, *Jour. Exp. Zool.*, 15: 527, 1913.

the melanophores. This method has proven reasonably successful.

It will be quite impossible to do more than discuss briefly the effects of some of the more important types of stimulation upon melanophores. These will be their responses to (1) variations in the electrolyte concentration of the medium, (2) changes in the amount and kind of radiant energy falling upon the cell, (3) responses to the presence of various drugs and (4) a short discussion of melanophore pulsations. In all this work the scales were removed from the trunk of the fish and their melanophore responses observed directly under the microscope.

ELECTROLYTES

Spaeth (1913) was the first to describe the effects of various electrolytes on pigment distribution. Very briefly his results may be summarized as follows. In solutions of sodium salts, as shown in Fig. 2, No. 6, the melanophores are completely dispersed. This dispersion lasts about three hours and is followed by a slow concentration. This last is a sign of the approaching death of the cell.

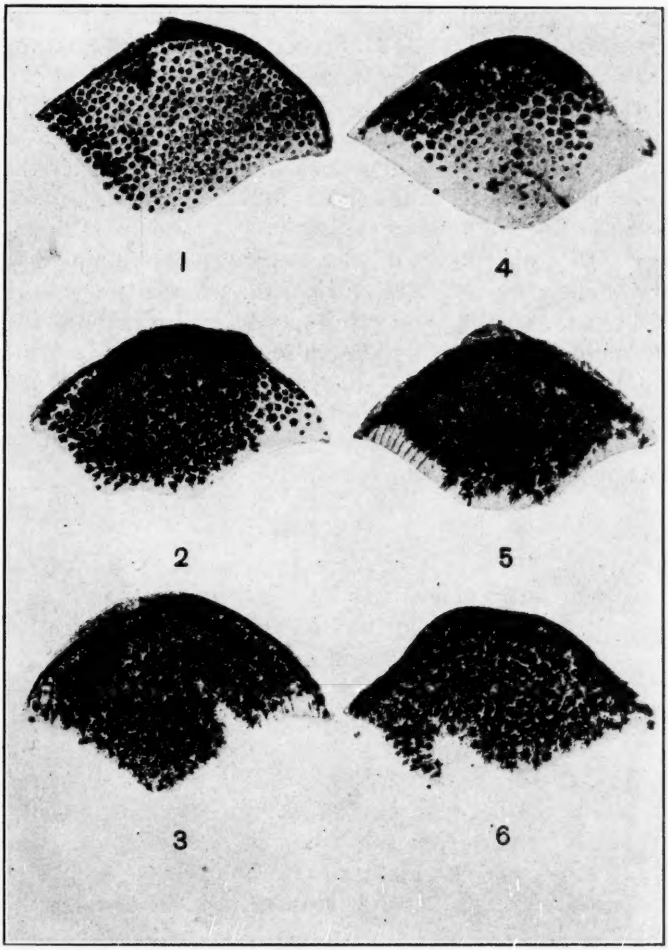

FIG. 2. Isolated scales of *Fundulus heteroclitus*, showing melanophores in different states of pigment aggregation. R. A. Spaeth, *Jour. Exp. Zool.*, 15: 527, 1913.

Spaeth found the expansion to persist longest in NaCl and to become progressively shorter as the anion shifted from Cl– to Br– to No^{3-} to SCN^{-}.

In solutions of potassium salts the melanophores become

completely concentrated, the scale resembling in appearance No. 1 in Fig. 2. The concentration lasts three or four hours and then is gradually replaced by a slight dispersion, again a sign of approaching disintegration. The period of concentration depends upon the anion and is longest in KCl, becoming progressively shorter as the anion is changed from Cl– to Br– to NO^{3-} to SCN^{-}.

As is to be expected, sodium and potassium salts antagonize each other, and the melanophores retain their irritability longer in a mixture of the two than they do in either one alone. As an artificial medium for maintaining melanophore irritability Spaeth found the best to be made up as follows: 6 vols. N/10 NaCl, 1 vol. N/10 KCl and 0.35 vols. N/10 CaCl2. This he called a "balanced solution," and with some modifications it has been used by most subsequent investigators.

RADIATION

Spaeth (1913) was also the first to show that on exposure to ultra-violet light (1,900–2,900 Å) the melanophores of isolated Fundulus scales showed a concentration of their pigment. This is shown in No. 1, Fig. 2. No such concentration occurs in control scales kept in NaCl nor in scales so placed that their bony portions lie between the melanophores and the light source (Fig. 2, No. 2). Apparently the radiation is absorbed in these cases before it gets to the cell. It takes from 7 to 10 minutes to complete the response and it is reversible in 20 minutes. The first cells to concentrate are those lying in the middle of the scale, and from here the reaction spreads towards the periphery. Spaeth's observations were later corroborated by O'Donnell (1927).

Spaeth, on the other hand, was unable to demonstrate any response to visible light in Fundulus scale melanophores. This is generally the case with most fishes. Some time ago, however, I was fortunate to observe a rapid dispersion of the melanophore pigment in Tautog scales when the light intensity was suddenly increased.

Fig. 3 shows this response, the scale having been kept for varying periods in relative darkness. The recording is by the photoelectric cell method previously described.

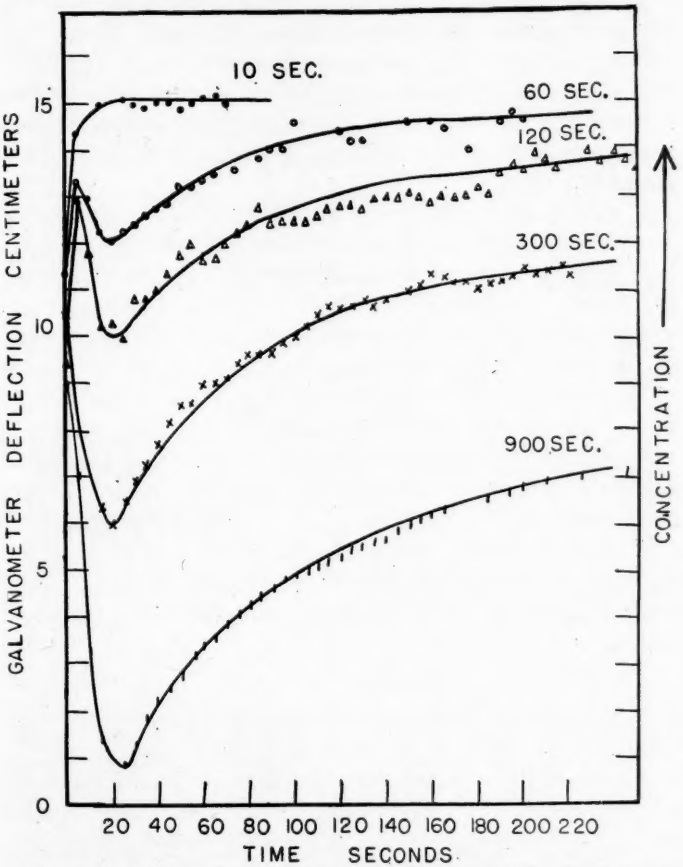

FIG. 3. Isolated scale melanophores of *Tautoga onitis*. Responses to sudden increase in illumination after varying periods in relative darkness. Photoelectric recording.

As can be seen from the figure, the dispersion is complete in about 30 seconds. Following this there is a slow return to the stellate or the concentrated state. The scales were kept in balanced solution throughout the experiment.

The extent of the dispersion seems to be proportional to the length of time the melanophores were dark adapted. Under the conditions of the experiment the response must be direct, since in an isolated scale there can be no question of circulatory or nervous mediation. It is my opinion that this response in Tautog scales is a persistent form of juvenile activity which is not retained in most fishes. No doubt it will be found in a few other forms if a search is made, although it definitely is not present in Fundulus scales.

Contrasting the responses of the scale melanophores to ultra-violet light and visible light, we note several marked differences. In the first place ultra-violet light produces a concentration and visible light a dispersion of the pigment. Secondly, the speed of the response varies greatly, being slow in ultra-violet light and very rapid in visible light. Finally in the irradiated scales the response appears first in the center and spreads outward, while in visible light the response occurs simultaneously throughout the entire scale. All this suggests that in the case of ultra-violet light the response is due to some chemical change in the tissues brought on by the radiation, while in the case of visible light it is due to direct stimulation of some presumably photosensitive material in the melanophore.

DRUG ACTION

It must not be assumed, however, that all responses of isolated melanophores are non-nervous in nature. There is the possibility that stimuli may act upon the torn ends of the severed nerves or upon the chromo-neural transmitting apparatus. The only way to exclude definitely the possibility of nervous intervention is to study the responses in scales in which all nervous structures have been eliminated. Following a method originally developed by Dr. H. B. Goodrich, such preparations may be obtained as follows. The scale is pulled from its scale pocket and all its connections with the body are severed. It is then immediately replaced in the pocket and in the major-

ity of instances it remains there. However, in conformity with the laws of nervous degeneration all the nervous structures in the scale should disappear in time. To see whether there are any changes in the melanophore responses which could be correlated with this nervous degeneration the reactions of the cells were tested from time to time to various stimuli. The most successful were those concerned with drugs.

Fig. 4 shows the results of such a study on the effects

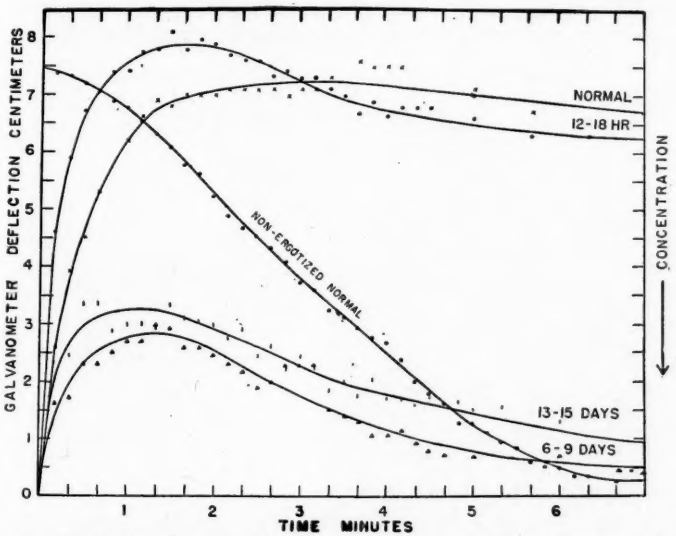

FIG. 4. Isolated scale melanophores of *Tautoga onitis*. Responses to adrenalin after previous treatment with ergotamine. Time after denervation of scale shown by figures above right-hand end of each curve. Photoelectric recording.

of adrenalin, both alone and after previous treatment of the scales with ergotamine. Adrenalin alone produces a marked concentration of the pigment. The figure shows such a response in a normal scale as recorded photoelectrically. No appreciable differences between the responses in normal scales and those taken at various times after denervation could be seen.

Such, however, is not the case with scales previously

treated with ergotamine. Spaeth and Barbour (1917) made the interesting discovery that after ergotoxine adrenalin expanded instead of contracted the melanophores. The same reversal may be produced in the Tautog scale with ergotamine, as shown in the figure. In about 24 hours after denervation, however, the character of the response is markedly altered and remains that way for at least fifteen days. While some dispersion still occurs, it is only a fraction of what it formerly was, and it persists for a relatively short period of time. Clearly the nervous degeneration going on in the scale has affected

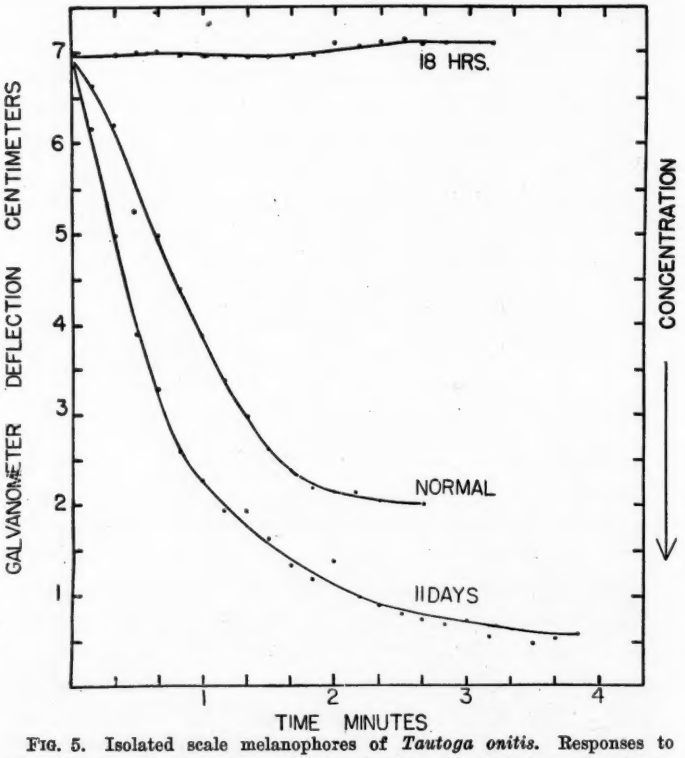

FIG. 5. Isolated scale melanophores of *Tautoga onitis*. Responses to N/5 KCl of normal scale and scales tested 18 hours and 11 days after denervation. Photoelectric recording.

some structure acted upon by ergotamine, which in turn is responsible for the reversal of the adrenalin reaction. Adrenalin alone still produces concentration at this time. In fact the only difference between this and the response to adrenalin after ergotamine is a slight momentary dispersion in the latter case. We may conclude from this that adrenalin acts at some point peripheral to the action of ergotamine. Since the ergotamine-adrenalin response is altered by nervous degeneration we may conclude that the response to adrenalin alone is direct. If adrenalin is the mediator of sympathetic impulses, as many think, such a result is not surprising.

Next the effects of KCl were studied. This also produces a marked concentration, as shown in Fig. 5. This concentration no longer occurs in about 18 hours after denervation, but reappears again in about 11 days. The concentration produced by KCl then depends upon the integrity of some structure associated with the innervation of the scale. From this we may conclude that KCl does not act directly upon the cell, but rather upon the chromo-neural transmitting apparatus, and therefore its action is proximal to that of adrenalin. Possibly KCl acts to release from the transmitting apparatus some adrenalin-like substance which in turn causes the pigment concentration.

Pulsations

Finally, in conclusion I want to mention briefly a very interesting type of response shown by certain chromatophores. These take the form of pulsations of the pigment granules and were first described by Ballowitz (1913) as occurring in the erythrophores of Mullus when immersed in NaCl. Fig. 6 shows the two extremes of pigment migration exhibited during these pulsations. The figure is taken from the original description by Ballowitz. We have also described similar pulsations in the scale erythrophores of the squirrel fish, Holocentrus (Smith and Smith, 1935). Spaeth (1916) quite independently observed a similar phenomenon in the scale melanophores of

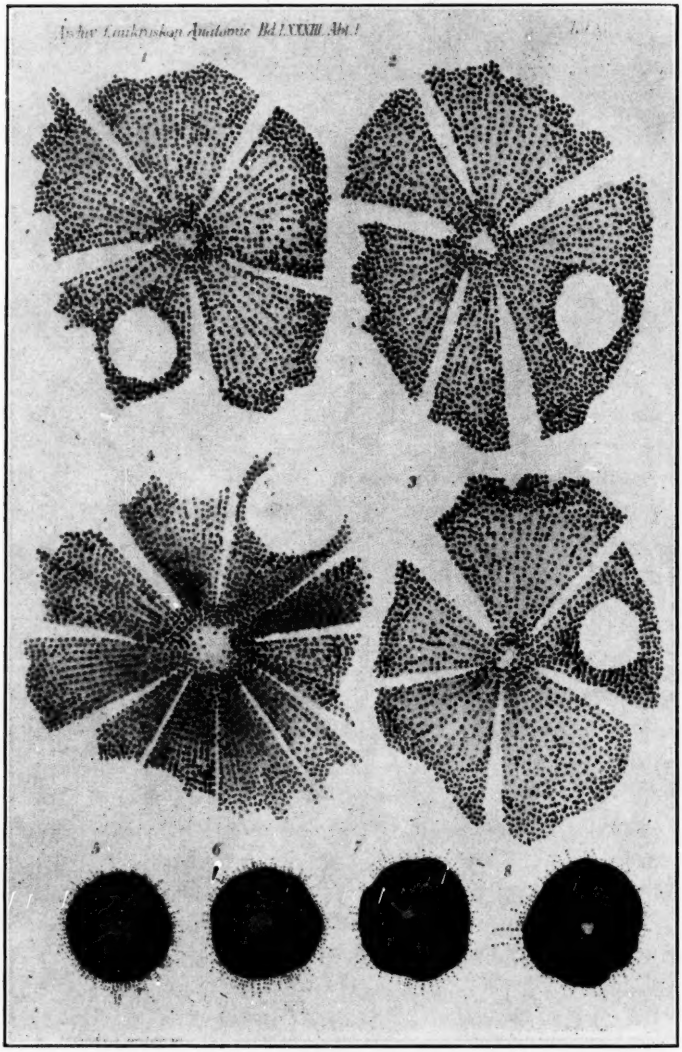

FIG. 6. The extremes of pigment distribution in the isolated erythrophores of Mullus during pulsations. E. Ballowitz, *Arch. mikro. Anat.*, 83: 290, 1913.

Fundulus immersed in NaCl after previous treatment with BaCl2. Fig. 7 shows the frequency and extent of these

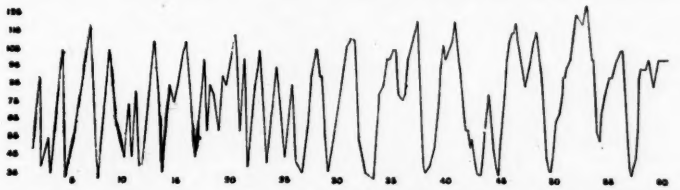

FIG. 7. Rhythmical pulsations in the isolated scale melanophores of *Fundulus heteroclitus* immersed in N/10 NaCl after previous treatment with N/10 BaCl2. R. A. Spaeth, *Jour. Exp. Zool.*, 20: 193, 1916.

pulsations as measured with a micrometer scale. These pulsations have been shown to vary in frequency and extent with the temperature, being more rapid and very slight at high temperature (30° C.) and much slower but with pigment migration over a greater distance at low temperatures (Smith, 1931).

Similar pulsations have also been seen in the scale melanophores of the Tautog. Their extent and frequency as measured with the photoelectric method is shown in Fig. 8.

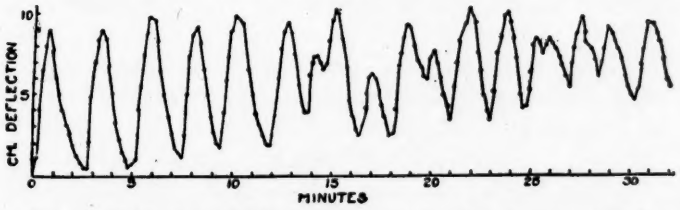

FIG. 8. Rhythmical pulsations in the isolated scale melanophores of *Tautoga onitis* (L.) as recorded photoelectrically. D. C. Smith, *Jour. Cell. Comp. Physiol.*, 8: 83, 1936.

Parker and Pumphrey (1936) showed that in Fundulus these pulsations were not dependent upon the presence of any nervous mechanism in the scale, and the same is undoubtedly true for other forms.

The nature of these pulsations is still obscure. They may be associated with some electrolyte disturbance in the cell. Yamamoto (1933), who studied them in the scales of

Oryzias, believes they are produced by an excess of sodium over calcium ions. In any event they are worth studying further, for they undoubtedly are an expression of the underlying forces producing pigment migration.

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HUMORAL CONTROL OF CRUSTACEAN CHROMATOPHORES*

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MANY higher crustaceans are able to change their color and pattern by activities of their chromatophores to accord more or less perfectly with the background upon which they reside. Generally speaking, the activities of the chromatophores in color changes are of two sorts: differential formation and destruction of the included pigments, and differential migration of the pigments within the chromatophores. This discussion will deal only with the controlling agencies of the pigment migration, but even with this restriction it will be impossible to include more than the principal facts, with the chief evidences for them.

The descriptive aspects of crustacean color changes were well advanced through the investigations of such men as Pouchet (1872–1876), Gamble and Keeble (1900–1905), Megušar (1908) and Degner (1912). These investigations were conducted during a period when the control of the color changes was believed to be wholly nervous. Complexity of response was then easily explained away in terms of central nervous phenomena. Degner had come very close to the modern concept of humoral control of chromatophores when he discovered that nerve transection failed to interfere with the chromatophore responses.

The real steps that revolutionized our outlook on the general problem came in 1925 and 1927 when Koller discovered that blood transfusions carried with them a substance or substances capable of inducing chromatophore response. In 1928 Perkins, working on Palaemonetes, and Koller on Crago [*Crangon septemspinosus* (Say)] independently reported that eyestalk extract had a very potent

* Read at a symposium on "Color Changes in Animals, Their Significance and Activation" at the annual meeting of the American Society of Zoologists in conjunction with the American Association for the Advancement of Science at Richmond, Va., December 29, 1938.

effect in inducing chromatophore pigment concentration. Perkins further demonstrated quite conclusively by means of exhaustive nerve transection experiments that the normal responses of the chromatophores to black and white backgrounds were in no way effected by direct nerve supply, but were totally controlled by blood-borne material. Koller, on his part, described the rostral region as a source of a second extract which worked antagonistically to the eyestalk substance.

Let us now look briefly at the pigmentary system and its activities to see just what we are attempting to explain. Crustaceans generally have three to five functional pigments within this system. The combination red, yellow, blue and white is common among shrimp. In certain other crustaceans a black or brown-black pigment is present.[1] Work already described has demonstrated the gross chromatophore control to be humoral. The extent of independent activity of the individual pigments will indicate to us the complexity of the situation which must be explained in humoral terms. Speaking of pigments, Keeble and Gamble (1900) say, "During colour-change they are distributed independently of one another in the sense that one pigment may become aggregated in the center of the 'chromatophore,' whilst another runs out into its network of processes. Change of colour appears to be due to a fresh pigmentary deal of the shuffled colour pack." Pigment independence in Hippolyte was confirmed by Minkiewicz (1908). Koller (1927) describes independence of the red and yellow pigments of Crago. Brown (1933, 1935a, 1935b) demonstrated that each of the four pigments of Palaemonetes was able to show independent activity. For all possible combinations of two pigments at a time, different experimental situations illustrated that one pigment could be dispersed or concentrated while the other pigment was simultaneously in the same or opposite state. Frequently the same pigment cells in different experimental situations were ob-

[1] This classification of pigments by color does not necessarily indicate chemical similarities.

served during several days and photographic records made demonstrating such independence. Observing the same pigment cells in such an experiment precludes the possibility that an apparent independence arises from the selection of different cells with different normal responses. More recently Abramowitz' (1935) data have demonstrated for the crab, *Portunus ordwayi*, an independence of the red and yellow cells.

Another interesting general behavior of the chromatophores should be stressed. There is a striking tendency for all the pigments in a crustacean, like Palaemonetes, to behave rather uniformly relative to one another. That is, in the great majority of the immediate responses of the chromatophores the red and yellow pigments concentrate or disperse together while the white usually migrates in the opposite direction. If one considered only this aspect of color change there would be but little evidence of an independent behavior of the various pigments, and the action of a single humoral substance could account for the whole. The delicate differential control of the pigments which occurs in the animal in response to the background color makes its appearance only after a period ranging from fifteen minutes to more than a day. It is this secondary migrational response that demonstrates the independence of the pigments. The investigations of Keeble and Gamble, Koller and Brown have all given indications of these two distinct types of pigmentary response. It would perhaps seem that the rapid initial response is determined by apparent brightness of background or change in total illumination. The secondary response of the pigment cells has apparently been overlooked by many recent investigators of crustacean chromatophore control. By finding quite normal responses in denervated areas of the body, Brown (1935b) showed that the secondary pigment migration demonstrating independent activity of the pigments was humorally controlled.

The recent extensive comparative surveys of color-changes and eyestalk-extract injection effects have con-

tributed relatively little to our understanding of the controlling mechanism of the intricate color adaptations in any single crustacean. They have, however, given strong suggestion that eyestalk-extract is qualitatively quite uniform regardless of its source among crustaceans.[2] It has demonstrated, furthermore, that chromatophores of the same color in different crustaceans may be inherently quite different in their responses. To the same eyestalk extract sample the red pigment of a brachyuran response by dispersion and that of a macruran by concentration. Until chemical isolation and purification of the constituent which induces concentration in one red pigment cell has been effected it is impossible to say definitely whether the same constituent or a different one is responsible for the dispersion in the red cell of another animal.

Hanström (1935, 1936) has presented good evidence that the active portion of the eyestalk in chromatophorotropic activities is the so-called sinusgland. This is composed of distinctly glandular tissue,[3] richly innervated, and present in all crustaceans so far examined.

No such general confirmation has been established concerning the rostral gland first described by Koller (1928, 1930), but in 1934 Beauvallet and Veil obtained data suggesting its presence in Palaemon. Although I am not prepared to give evidence in favor of a functional rostral gland, I do have some observations upon *Crago septemspinosus* which indicate a definite difference in the control of some of its chromatophores from that found in Palaemonetes. The observations were as follows: Crago and Palaemonetes failed to respond in the same manner to removal of both eyestalks with their included glands. Although Crago and Palaemonetes both darkened maximally at first, Crago finally assumed a mottled condition while Palaemonetes remained dark. Artificial stimulation of the stubs of the excised stalks produced transitory

[2] This may not be true from the standpoint of retinal pigment controlling hormones (Kleinholz, 1936).

[3] The cells show eosinophilic cytoplasmic inclusions.

darkening in Crago and transitory lightening in Palaemonetes. In another experiment, occluding the blood supply to the eyestalks and rostral region resulted in rapid darkening in light-adapted Palaemonetes, but almost no darkening in Crago, during the hour following the occlusion. These observations find a reasonable explanation in the activity of a dispersing hormone, perhaps of rostral origin, in Crago and the absence of one in Palaemonetes.

There is no evidence beyond these few cases already mentioned suggesting the presence of a humoral substance operating antagonistically to the eyestalk extract. On the contrary, in the majority of the crustaceans examined, there is evidence pointing to the absence of such substances. Procedures rendering the eyestalk glands ineffective are conducive to chromatophores assuming a condition opposite that which has been shown to be the result of an activating humoral substance. For example, general red and yellow pigment dispersion follows removal of the eyestalks, or occlusion of blood supply to the eyes. Cutting an artery results in pigment dispersion in the region devoid of blood supply. Isolation of pieces of integument results in similar behavior of the isolated pigments. A very significant experiment was performed by Perkins and Snook (1931), who produced repeated concentration and dispersion in the red and yellow cells of an isolated piece of integument merely by adding eyestalk extract. The chromatophores showed dispersion of their pigments as the effectiveness of the extract disappeared. Even the rates of dispersion resulting from isolation are approximately those seen in normal responses (Brown, 1935b).

There are a number of indications of the production of chromatophorotropic substances in regions of the body outside of the eyestalks. Such evidences come from injection and eyestalk extirpation experiments. Injections of extract of central-nervous-organs induce concentration of shrimp red and yellow pigments. This was independently shown by Brown (1933) and Hosoi (1934). The activity of the extract is independent of the state of adaptation of

animals from which the extract was made. Eyestalkless crustaceans show activity of their chromatophores. This activity may be random as with the white cells of some forms, in response to artificial stimulation of the eyestalk stubs, or in response to changes in light intensity. By means of such experiments and study of the effects of injection of the crude extracts of eyestalks we are led to the conclusion that the chief source of the red and yellow pigment controlling agents is in the eyestalk, while the chief source of the white pigment controlling substance is outside of the eyestalk. Further evidence of the extra-eyestalk origin of pigment controlling substances comes from study of the quantitative effects of the potency of single stalks as compared with two stalks in the normal responses.

Except in the instance of Crago, where antagonistic hormones may control a single pigment, a single type of effect of an activating substance upon a pigment seems to be the rule. Originally we believed this effect to be always pigment concentration, but since the work of Carlson (1935) we know that it may be either pigment concentration or dispersion, depending upon the species. The reverse response appears to result passively from the disappearance of the activating substance.

To account for the apparent independence of pigments Brown (1935b) has postulated the existence of a separate hormone for each pigment of Palaemonetes. This seemed to be the most reasonable explanation in that it requires the fewest hormones. If one assumes that eyestalk extract contains a single homogeneous chromatophore activator, then one would need to postulate the addition of a minimum of four "modifiers" to explain the normal pigment independence in color changes. An interesting speculation may be advanced here, namely, that the sinusglands secrete a chromatophorotropic substance the molecules of which are subject to modification of superficial structure through nerve control. Such molecules might find their most stable form or equilibrium proportions in the condition seen in eyestalk extracts.

The rich nerve supply to the sinusgland from the lamina terminalis described by Hanström is interesting in connection with any one of these hypotheses. Suggestive also is the condition of eyestalk extract in such forms as Cambarus in which the chromatophorotropic activity is greatly enhanced by boiling. It is possible that boiling may remove inhibitors of chromatophorotropic activity. A comparable but more selectively inhibiting mechanism might be operative in the "four-modifier" hypothesis mentioned before.

Abramowitz (1937) speaks of the hypothesis of the system controlled by several hormones as the "multiple theory." He proposes as a possible alternative a "unitary theory" and states that it explains the situation equally well. The unitary theory is contradicted by certain facts. For instance, it has been shown that the red and yellow pigments may show degrees of concentration and dispersion quite independent of one another. To explain this in terms of a single hormone would require differences in threshold of response for the two pigments, and furthermore, there would need to be some higher concentration of the hormone which would simultaneously induce dispersion of one pigment and concentration of the other. Experimentally, no injection of eyestalk extract, if above the minimum strength required to concentrate both pigments, has resulted in dispersion of one of them. This has been true though very concentrated extracts have been used. Similarly, a unitary theory is even less able to explain the relatively independent behavior of four pigments. To do this last, some pigments must have not only a single threshold value of hormone concentration for activation but must have a number of alternately activating and non-activating values as the hormone concentration is gradually increased.

Since some chromatophores in a number of crustaceans appear to respond to a greater or less degree to direct light stimulation it might be suggested that this fact, together with a single hormone, may explain the color adaptations.

This is highly improbable, since blinded animals show no longer any trace of background response and also because the independent behavior of the pigments can be demonstrated in the dorsal trunk region cells where the illumination on the cells is almost exclusively the direct light in contrast with the background reflected light. This direct light may be maintained constant in quantity and quality in experiments demonstrating pigment independence.

Finally, we know nothing whatsoever of interactions and mutual effects of the various hormones operating in color changes. This alone could complicate the picture considerably.

Summarizing, it may be said that the crustacean chromatophore system contains four or five independently acting and humorally controlled pigments. These facts point to the functioning of at least four, and possibly more, hormones. These substances appear to be formed chiefly in the sinusgland of the eyestalks, though secondary sources may be present. The pigments appear to have one phase of their activity, concentration or dispersion depending upon the species, determined by action of a hormone, while the remaining phase appears to be the result of absence of the hormone. For only one crustacean, Crago, does evidence remain suggesting dual control of a pigment.

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DISTRIBUTION OF INTERMEDIATE-WINGED APHIDS IN THE FAMILY AND ITS BEARING ON THE MODE OF THEIR PRODUCTION[1]

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THE method of stimulation of wing development in aphids may presumably be either induction or the influence of some hormone or other chemical substance. Induction would involve the action of cells or tissues on other cells or tissues with which they are in contact or at least in very close proximity, and the spread of the stimulus should be relatively slow. A hormone could be in the body fluids, and so spread rapidly to any part of the organism, far as well as near.

As between these two methods, the conclusions of Stiles (1938) regarding the order of determination of the several distinguishing structures of winged aphids appear to favor the latter. Stiles obtained evidence that, of the three chief features, wings are determined first, then the ocelli, then the wing muscles. Wings get their start in some way not yet discovered. If the ocelli and wing muscles owe their stimulation to induction from the wings, that influence could either be a chain in which wings stimulate ocelli and ocelli stimulate wing muscles, or the wings could induce both ocelli and wing muscles directly, the former earlier than the latter. If induction is a chain, it acts in a roundabout way, spreading from wings to the more distant ocelli, then returning to the wing muscles, which closely adjoin the wings. If induction is a direct influence of wings on both ocelli and wing muscles, it spreads the greater distance to ocelli faster than the shorter distance to wing muscles.

Though these considerations are not conclusively against the induction theory, they render the hormone theory the

[1] Contribution from the Department of Zoology of the University of Michigan.

more probable. The hormone, while presumably produced at some one place, would quickly be everywhere, through circulation, ready to act as soon as the conditions of stimulation and of response were met.

How does this conclusion bear on the occurrence of intermediate-winged aphids? If it be assumed that the production or suppression of wings is dependent upon the presence of a hormone in a given concentration at some critical time in development, and if this substance may be accumulated at different rates or in different total amounts so that intermediate-winged individuals are sometimes produced, then it would be expected that some relation would exist between the frequency of intermediates and the frequency of fully winged aphids. Various possible mechanisms, whose operations would lead to the development of intermediates or mosaics or both, have been discussed in an earlier paper (Shull, 1937). A greatly simplified modification of the scheme will suffice for our present purpose.

The adopted scheme owes some of its characteristics to the discovery that all aphids of the species studied (*Macrosiphum solanifolii*) have thickened hypodermis in the wing-bud region in embryonic stages before birth (Shull, 1938). Even those which will be wingless as adults have wing buds before they are born. Something must stimulate that thickening. The wing buds disappear later in those that are to be wingless, but increase their thickness and begin to bulge in the prospective winged ones.

One possible assumption is that all embryos contain a hormone in sufficient concentration to stimulate observable thickening of the hypodermis. A greater concentration is needed, however, to cause further growth, and this concentration must be present shortly before birth when wings are finally determined. Only in certain individuals or under certain conditions is the concentration thus increased early enough; hence some aphids remain wingless. Figure 1, A, illustrates this scheme. If in some individual the concentration of the hormone reaches a level between

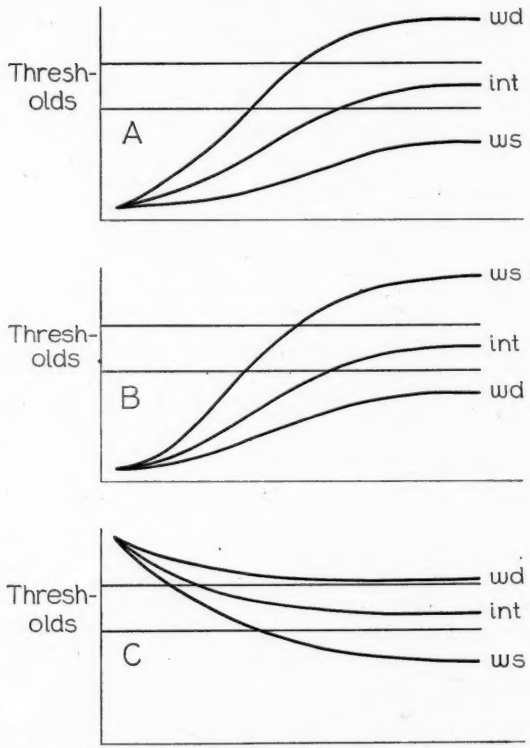

FIG. 1. Diagrams of several possible schemes by which wing development may be stimulated or inhibited by a hormone. The heavy curves represent the concentration of the hormone. The light horizontal lines represent the concentrations which will stimulate beginning development (lower) and complete development (upper), respectively. A, stimulating hormone; B, inhibiting hormone; C, stimulating hormone which decreases in amount with age.

that sufficient to stimulate the beginning of wing development and the amount necessary to cause complete development, intermediate wings are produced. The existence of intermediates necessitates the assumption that there is a gap between these thresholds of stimulation or that determination of wings is spread over a time interval. The latter possibility is not illustrated in this diagram, but was considered in the earlier paper (Shull, 1937).

Another possibility is that the hormone is inhibitory in its effect. The initial thickening of the hypodermis might be supposed to be due to some other agent, perhaps another hormone. Prevention of wing development would occur only in individuals in which the inhibiting hormone reached certain concentrations (Fig. 1, B). High concentrations would thus result in winglessness, not in wings as in A. Intermediates could again be produced by moderate concentrations or a spread in the period of determination.

It is not necessary to assume that the hormone will increase in amount; it may diminish. A concentration sufficient to cause wing-bud thickening may be present in all embryos, but in some individuals under certain conditions become diluted later so that further development is not stimulated (Fig. 1, C).

These examples will suffice to illustrate the supposed action of the hormone. Other schemes are possible.

Altered Rates of Hormone Production as Source of Intermediates

It has generally been assumed, on any scheme like the foregoing, that intermediates owe their existence to modified rates of production of the stimulating or inhibiting hormone. Any rate which brings the concentration to some level between the thresholds of beginning and of complete development, at or before the critical time of determination, should lead to intermediacy. On this hypothesis, the several postulated schemes of hormone action illustrated in Fig. 1 should produce results having one thing in common, namely, a relation of frequency of intermediates to frequency of winged individuals. The reasons are briefly the following.

If embryos start development as of one morphological type, and if under given conditions many but not nearly all of them finish as of another type, numerous embryos must have crossed the threshold or thresholds separating the two types in development. The histology of prenatal hypodermis in aphids suggests that the development is at

first always toward the winged condition, but the argument may be made wholly general. If all the aphids are of one type in the adult stage, all of them may have crossed the thresholds, or none of them may have crossed. Conditions producing such a result are not very illuminating. When, however, the end results include both types in considerable numbers, many individuals must have crossed the developmental thresholds no matter what scheme of hormone control is in operation.

In the crossing of these thresholds lies the opportunity to produce intermediates, according to the theory involving rate of hormone production. The more individuals which have crossed them, the more intermediates there should be, other things being equal. If crossing the thresholds stimulates wings, the frequency of intermediates should be positively correlated with that of winged aphids. If crossing the thresholds inhibits wings, the frequency of intermediates should be negatively correlated with that of winged aphids.

PREVIOUS INDICATIONS OF CORRELATION OF INTERMEDIATE WITH WINGED APHIDS

In certain groups of earlier experiments with the strain of aphids collected in Ann Arbor in 1923, it appeared that "in general the greatest production of intermediate wings does not accompany the greatest production of wings" (Shull, 1937). In those experiments "the correlation between the two is found to be $r = -.68 \pm .10$"; that is, "the fewer the wings the more numerous the intermediate wings." In another strain of aphids, however, "the greatest number of intermediate aphids would seem to be produced under circumstances which favored medium wing production. . . ."

The conclusions just quoted, which were not the primary goal of the previous studies, were vitiated somewhat because only total numbers of winged and of intermediate aphids in each family were used. An appearance of negative correlation might be created by a very strong genuine

negative correlation in one part of the family together with no correlation or mildly positive correlation in another part. Such a condition would scarcely indicate a real connection between the two classes of aphids.

Distribution of Intermediates in the Family

The distribution of both intermediate and winged offspring in the family has now been ascertained for aphids reared in environmental conditions which produce enough intermediates to make a reliable comparison. Only the 1923 strain of aphids was used because the 1931 strain produces too few intermediates in almost all conditions. The distribution was ascertained by transferring the parents to a new plant every two days (if at 24°) or every five days (if at 14°). The offspring produced on each plant were reared as a group and the number of winged and intermediate-winged among them determined. Usually from six to eight successive plants were required to include practically the entire family.

The results of these tests are presented in Table 1. The letters *a, b, c,* etc., designate the successive plants, hence the successive groups of offspring. All aphids produced on plant *a* of the several families are combined into one total, all those on plant *b* of the several families into another total, and so on. The total number of aphids of all kinds (including the gamic forms which were uncommon) is given for each plant, and the winged and intermediate-winged ones are given as percentages of these totals. If it is desired to know the actual numbers of individuals, these must be calculated from the total and percentages. Too much attention should not be paid to the last groups of offspring in most experiments, for the total numbers of aphids are so small that percentages may well rise or fall in meaningless ways.

The percentages of winged and of intermediate-winged offspring in this table are graphically shown in Fig. 2. Within each rectangle are shown the winged and intermediate aphids in the experiments carried on in one set of

TABLE 1

SHOWING THE DISTRIBUTION OF THE WINGED AND INTERMEDIATE OFFSPRING IN THE FAMILIES OF DIFFERENT KINDS OF PARENTS REARED AT VARIOUS CURRENT AND ANTECEDENT TEMPERATURES. ALL PARENTS WERE REARED IN CONTINUOUS LIGHT DURING THE EXPERIMENT. THE COLUMNS HEADED *a*, *b*, *c*, ETC., INCLUDE SUCCESSIVE GROUPS OF OFFSPRING FROM THE BEGINNING OF THE FAMILY TO THE END

Type of parent	Temperature at which: Parents' ancestors were reared	Parents were reared to 4th instar	Parents were kept from 4th instar on, and offspring were born	Offspring on successive plants: Types of data \ Plants	a	b	c	d	e	f	g	h
Wingless	24°	24°	24°	Total	1967	1158	1160	836	505	142	5	
				% Winged	55.52	23.58	13.02	18.90	25.74	21.83	0.00	
				% Int.-wd.	1.63	3.38	2.67	2.51	5.74	20.42	20.00	
			14°	Total	1314	1291	1091	967	786	142	1	
				% Winged	42.92	3.49	4.22	12.62	18.96	2.11	0.00	
				% Int.-wd.	3.12	2.25	3.94	6.83	12.60	7.75	0.00	
	14°	14°	24°	Total	1528	1091	942	760	701	218		
				% Winged	56.28	37.58	36.73	48.55	53.78	55.05		
				% Int.-wd.	2.16	3.76	5.10	9.61	9.13	11.47		
			14°	Total	890	1046	1105	1010	922	310	23	
				% Winged	56.97	14.72	19.10	15.45	4.88	2.88	0.00	
				% Int.-wd.	3.71	7.17	11.13	12.67	10.95	4.52	4.35	
	Alt.	Alt.	24°	Total	1844	1081	977	904	576	165		
				% Winged	60.63	30.53	14.84	26.44	41.84	49.09		
				% Int.-wd.	1.52	2.96	2.56	2.54	5.21	15.15		
			14°	Total	1219	1146	1199	1007	798	307	26	
				% Winged	41.67	3.66	5.17	7.45	6.64	0.00	3.85	
				% Int.-wd.	3.77	2.27	9.76	12.51	14.66	5.21	3.85	
	Alt.	Room	24°	Total	827	589	569	386	216	104	18	
				% Winged	62.88	19.69	14.41	21.24	44.44	55.77	22.22	
				% Int.-wd.	1.21	2.21	2.28	4.40	6.02	12.50	16.67	
			14°	Total	555	585	538	523	328	184	47	
				% Winged	60.36	3.25	2.04	11.85	14.94	8.70	0.00	
				% Int.-wd.	2.70	1.37	5.02	9.94	25.61	25.00	4.26	
	Room	Room	24°	Total	1321	881	687	530	343	193	4	
				% Winged	70.25	36.66	15.87	16.04	27.70	43.52	25.00	
				% Int.-wd.	0.38	4.99	3.06	2.45	6.12	5.69	0.00	
			14°	Total	657	717	604	438	389	220	23	
				% Winged	52.66	2.37	1.16	2.97	3.34	13.18	0.00	
				% Int.-wd.	3.65	2.79	2.48	4.34	14.40	17.73	13.04	

TABLE 1—(*Continued*)

Type of parent	Temperature at which: Parents' ancestors were reared	Parents were reared to 4th instar	Parents were kept from 4th instar on, and offspring were born	Offspring on successive plants: Types of data \ Plants	a	b	c	d	e	f	g	h
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			14°	Total	657	717	604	438	389	220	23	
				% Winged	52.66	2.37	1.16	2.97	3.34	13.18	0.00	
				% Int.-wd.	3.65	2.79	2.48	4.34	14.40	17.73	13.04	

TABLE 1—(*Continued*)

Type of parent	Temperature at which: Parents' ancestors were reared	Parents were reared to 4th instar	Parents were kept from 4th instar on, and offspring were born	Offspring on successive plants: Types of data \ Plants	a	b	c	d	e	f	g	h
Winged	24°	24°	24°	Total	2029	1398	1372	1013	679	244	42	
				% Winged	37.41	35.55	26.02	42.45	69.81	48.77	71.43	
				% Int.-wd.	2.51	4.58	3.35	4.64	5.89	9.84	0.00	
			14°	Total	3144	3804	3780	3164	2706	527	219	
				% Winged	26.27	12.57	21.11	20.04	18.07	12.33	19.18	
				% Int.-wd.	2.77	4.23	8.10	8.69	8.06	8.16	5.02	
	14°	14°	24°	Total	2999	2019	1153	411	268	140	37	
				% Winged	16.11	26.94	29.84	33.58	45.15	51.43	27.03	
				% Int.-wd.	1.00	1.58	3.90	9.49	15.30	6.43	21.60	
			14°	Total	1058	997	839	651	380	137	22	
				% Winged	14.46	11.42	10.25	14.13	6.32	0.00	0.00	
				% Int.-wd.	2.36	8.24	15.26	24.88	18.16	10.95	4.55	
	14°	Room	24°	Total	894	743	591	425	332	234	101	5
				% Winged	40.83	22.61	16.07	19.76	30.12	48.29	61.39	100.00
				% Int.-wd.	1.68	1.48	3.05	1.18	3.61	7.26	6.93	0.00
			14°	Total	544	735	577	550	354	335	74	
				% Winged	29.23	4.76	7.28	7.45	6.50	7.76	2.70	
				% Int.-wd.	2.39	2.86	10.23	13.82	21.47	9.85	13.51	
	Alt.	Alt.	24°	Total	2677	1885	1489	1401	1072	584	102	37
				% Winged	41.73	29.92	36.20	50.82	66.04	56.85	63.73	70.27
				% Int.-wd.	2.09	3.71	4.78	5.35	5.50	8.39	3.92	8.11
			14°	Total	1619	1881	1626	1636	1474	660	45	40
				% Winged	23.04	8.40	13.47	10.64	12.08	7.12	2.22	10.00
				% Int.-wd.	3.03	7.18	13.53	16.32	14.52	13.64	20.00	15.00
	Alt.	Room	24°	Total	938	716	664	475	327	293	98	34
				% Winged	21.75	13.83	20.78	21.26	32.72	57.68	48.98	94.12
				% Int.-wd.	0.11	0.98	2.71	2.11	6.73	3.41	3.06	2.94
			14°	Total	548	611	624	517	442	331	89	53
				% Winged	10.40	2.78	5.77	11.03	8.14	7.55	8.99	0.00
				% Int.-wd.	1.64	2.13	6.25	14.31	13.80	18.13	13.48	0.00
	Room	Room	24°	Total	2082	1352	1343	1013	799	394	32	
				% Winged	42.65	25.59	17.80	23.69	38.30	56.09	31.25	
				% Int.-wd.	3.03	2.51	2.98	4.74	7.51	13.20	6.25	
			14°	Total	1285	1422	1494	1300	1158	571	34	
				% Winged	28.33	3.94	7.50	8.23	9.15	7.01	2.94	
				% Int.-wd.	2.57	2.81	7.90	16.92	18.22	12.43	2.94	
	?	?	14°	Total	2057	2579	2790	2177	1981	556	312	
				% Winged	28.25	13.88	17.24	18.19	13.58	14.93	14.74	
				% Int.-wd.	3.31	3.84	5.56	6.11	5.40	6.29	5.13	

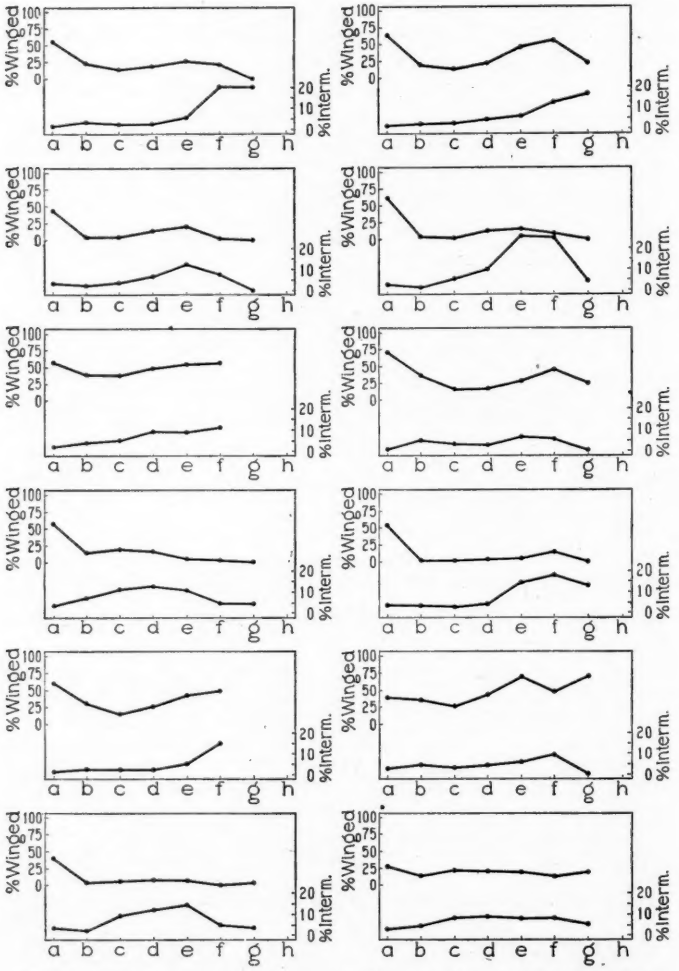

FIG. 2. Curves representing the percentages of winged and intermediates in the successive groups of offspring, based on the data of Table 1. Each rectangle represents one of the 23 groups of parental characters and treatments in that table.

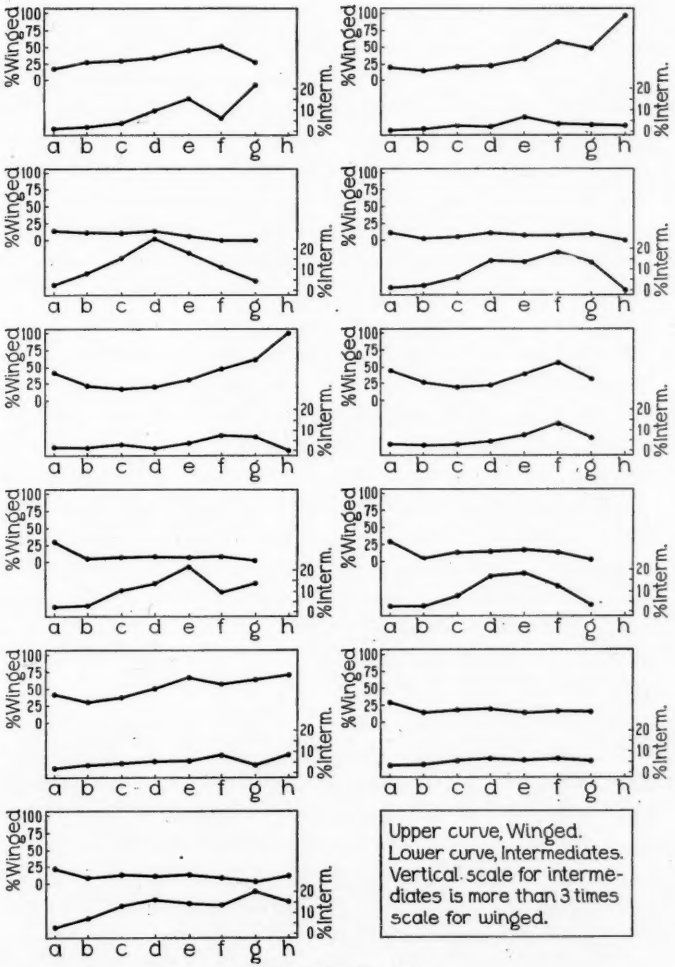

FIG. 2 (*Continued*).

conditions with parents of a given nature and origin. The 23 rectangles thus refer to the 23 trios of lines in Table 1. The upper curve in each represents the winged offspring, the lower curve the intermediates. The curve of intermediates is drawn to a larger scale than that of the winged, in order to discriminate more accurately between the generally lower percentages of intermediates. No confusion is likely to result from this difference in scale.

RELATION OF WINGED APHIDS TO INTERMEDIATES

The curves show a very strong tendency for the winged offspring to be numerous at the beginning of the family. In most of them the percentage of winged offspring on the first plant is never again equaled on any succeeding plant. Even where the later *percentages* are greater than the first one, they are usually based on total numbers so much smaller that the actual numbers of winged aphids late in the family is smaller than the early ones. When, therefore, total numbers of the two kinds in the family were alone considered, as in the former study (Shull, 1937), the percentages were largely determined by the percentages early in the family. Since the early groups of offspring included on the average many winged ones and few intermediates, a negative relation (somewhat less striking) was imposed upon the totals.

This negative relation is not clearly indicated by the later groups of offspring. Sometimes there is an opposite trend of the two curves, as in the first rectangle where, toward the end of the family, the percentage of winged offspring declines while that of intermediates rises. A similar opposition in greater detail is observable in the last two groups of offspring in the eleventh rectangle. Sometimes the two curves are similar (after the first lot of offspring), as in the second pair of curves and less strikingly in the 8th and 10th pairs. More often, however, the crests and troughs of one curve appear to be placed quite irrespective of the crests and troughs of the other.

There is one possible weakness in the present data, in

that the offspring borne on each plant were usually derived from several parents. At the outset 6 or 8 parents were put on the plant; these gradually died until the last plant often bore only one or two. It is conceivable that the intermediate-winged individuals were produced by one or two of the parents, and the winged offspring by the other parents; or the winged and intermediate offspring by certain parents, wingless ones by other parents, and so on. Such a condition would mean, however, that each aphid was more or less a law unto itself in its developmental response to light and temperature. While the scheme of developmental control has been proved to be very flexible with respect to the differentiating characters of gamic and parthenogenetic females (Shull, 1933), with numerous modifications of it in individuals, nothing so erratic has been found as would be involved in the assumption that individual differences would create the appearance of correlation where none existed, or destroy the signs of correlation where it actually occurred. The experiments would have to be conducted in a much more laborious manner to eliminate any conceivable error from this source. In the meantime it is assumed that the results mean what they appear to mean.

Another Possible Source of Frequency of Intermediates

Since the frequency of intermediates is not clearly a function of the frequency of winged aphids, other relations must be sought. It will be observed from the curves that the peak of production of intermediates, if there is a peak, occurs late in the family. In two of the curves it is about the middle, in all the others in the latter half. In no group are there more intermediates in the early part of the family than at any later time.

These facts indicate some dependence of intermediates upon age. To all appearances it is the age of the mother that thus governs frequency of intermediates among her offspring. It would be possible, however, to regard the

aging as occurring in the protoplasm of which the offspring are composed, since oöcytes must be nearly contemporaneous in their origins and some of them are long delayed in their conversion into embryos. It is impossible to decide at present where the age effect occurs.

The nature of this age influence can only be conjectured. Certain probabilities may be assumed, however, regarding the mechanism through which it modifies the frequency of intermediates. The absence of a clear connection between numbers of intermediates and numbers of winged or wingless indicates that this mechanism does not involve a change in the rate of production of the stimulating hormone such that the curve of concentration of the hormone crosses the thresholds of wing production or wing inhibition either more or less frequently.

The remaining sources of more intermediates, which are in harmony with the prevailing concept of developmental control, are a spread between the concentration which will initiate wing development and that which will insure its completion, and an extension of the period of determination. Either of these would suffice, but both could operate simultaneously.

The spread of concentrations of the hormone would appear to have the larger range open to it, and is accordingly favored. Such a spread should be at least as liable to modification by age as would length of a period of determination. It is possible, also, though hardly probable, that the age effect is in some degree comparable to the influence that arose in an old parthenogenetic line after six years of laboratory culture (Shull, 1932), when it reversed some of its differential responses and rendered them at the same time less definite. The validity of this comparison would depend on whether age effects could pass over from one generation to the next through the egg or through some cumulative maternal influence on the embryos; and it is impaired by the fact that the old parthenogenetic line changed the frequency of its production of intermediates rather suddenly instead of gradually.

The speed with which old lines change their responses is under investigation in another connection at present.

Summary

Previous work indicated that, as judged from the total number in the family, there is a negative correlation between the frequencies of winged and intermediate-winged aphids. This could be interpreted to mean that intermediates result chiefly from changed rates of production of a stimulative (or inhibiting) hormone. When the distribution of intermediates and of winged aphids in the family was studied, however, it was found that no such regular relation existed. The apparent correlation arose from a strong tendency to produce winged offspring at the beginning of the family, at which time the intermediates were always few. Intermediates are in some degree an accompaniment of age. As such, they are more probably dependent on a wider spread between the thresholds of beginning and complete development of wings, or on a lengthened period of determination of wings, in older parents, rather than on changed rates of hormone production. The spread between thresholds is favored.

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COMMENSALISM AND DOMESTICATION[1]

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I

Commensalism is a close partnership on equal terms between two individuals or groups of individuals. The partners do not influence each other, but simply live together under the same general conditions of environment. This forms a contrast to conditions obtaining under symbiosis on the one hand and parasitism on the other. Symbiosis, like commensalism, is a partnership on equal terms. But the partners influence one another profoundly, and are not able to exist without each other. Parasitism, on the contrary, is an unequal partnership. The parasite itself is not able to exist outside the host; he lives off the host and is injurious to him.

The commensals which it is proposed to discuss in this connection are all mammals, and they are all commensals of man. They include a mouse, 4 species of rats and a shrew. It has been suggested that these animals are not really commensals, but that they are parasites. But it it quite obvious that from the biological point of view it does not matter whether they sit at man's table or whether they live off his table. The whole idea, it seems to me, is adapted from human conditions, and the difference suggested may have an economic or a social background, but it has no biological meaning at all. As the main features pointed out to be characteristic of the commensal, namely, loose partnership under the same conditions of environment with man, apply to these animals, there is no reason to regard them as anything but commensals.

II

I shall now give a short description of the species we are going to deal with.

[1] Lecture, Biological Laboratory, Harvard University, January 21, 1938.

The first of them is the house-mouse, *Mus musculus* L. This is now a cosmopolitan animal. Its origin, ancestry, evolution and history are well known and will be discussed in detail later on.

The second species is the house or black rat, *Rattus rattus* L. The origin and the ancestral type of this animal are known in a general way, but in a general way only. What can be said with certainty is that the house-rat has been developed from a species of rat found wild in the Malay area; but we do not know yet the subspecies, nor have we been able to form any idea whether one or more than one subspecies are ancestral to the commensal, that is, whether the commensal is monophyletic or polyphyletic. We also may regard it as a proved fact that the actual differentiation of the commensal has been effected in continental India and that migration has begun there. From India the house-rat has reached the Mediterranean and Europe over land, on the trail of Neolithic man, and its world-wide distribution has had its center in Europe. This animal is largely transported by shipping across the ocean and along inland waterways, like the Congo or Amazon. Its routes of migration can now be largely reconstructed.

The lesser Malay house-rat, *Rattus concolor* Blyth, is our third type. This species is not cosmopolitan. It is restricted to the Malay area and Oceania as far as Hawaii and New Zealand, where it is the common house-rat. Its origin must be Malayan, although a connection between the commensal and the wild stock has not been established. In fact, we are not quite certain what the wild stock is like.

Another member of the genus *Rattus* has become as important and ubiquitous a commensal as the house-rat; indeed, being the stronger of the two, has replaced it in many places. This is the brown, or Norway rat, *Rattus norvegicus* Berkenhout. The actual wild ancestor, as in the lesser Malay rat, remains to be established. But the species as such can be traced back as far as southeastern China, from where it has spread to the north and west

and has reached Europe on the northern transcontinental route. From there, like the mouse and the black rat, it has spread elsewhere.

The East African Negro rat, *Mastomys coucha* Smith, it not very widely known. It is found all over Africa, and it has a tendency to live in Negro huts. Like other commensals in close connection with man it has developed identical changes, chiefly in color. Although its distribution is limited, it has become important, as, like the house-rat, it carries plague.

The last commensal to be dealt with, the large Indian musk shrew, *Suncus caeruleus* Kerr, is not a rodent but an insectivore. The fact that this is a commensal is not generally known, indeed, the connection between the wild stock of continental India and the various commensal types has not been worked out at all. Most of them have been described as separate species or subspecies, and that was all. But there can not be any doubt that we have to deal with a true commensal. We know that it lives in houses and native house-boats, and that it has been carried by man. Its present distribution, in addition to continental India, includes the whole of Malaya and southern China, and it is also found all along the east coast of Africa and the shores of the Red Sea.

III

The question may be asked why all these animals are regarded as commensals, and not as wild animals which have looked for shelter and food in houses and other human settlements, and run wild again if and when they want to. This is true of early commensal types, but it is not so in more advanced commensals which have developed changes under commensal conditions that make them fitted for an indoor but less fitted for an outdoor life.

IV

The changes produced by commensal life are various. They affect the type of color, and the control the pigment itself. They affect the general size of the body, but also

that of its various parts, the length of ears, of feet, of the tail. They are evident in the shape of the skull, in particular one change, the reduction of the length of the face compared with that of the brain case. These changes may be transitory, non-inheritable, and may disappear in the offspring. But the true commensal characters are real mutations. Why and how they appear we shall see later on.

V

The commensal best known at present is the house-mouse. During the last two years I have myself had an opportunity to do some research on this species. Almost everything is now known of its ancestry, evolution and history. It is, therefore, that I shall try to point out the conditions that lead to commensalism, and to explain the changes that are typical in the development of commensal characters, on the evidence of the results obtained in the study of this animal.

The house-mouse group as it now exists is not a homogeneous crowd. There are three wild ancestral stocks representative of three different subspecies of the species *Mus musculus*. All the house-mice of East Central Europe and of European and Asiatic Russia have sprung from a wild stock of their own, and the same is true of the Japanese house-mouse. But all other house-mice in the world, whatever the local type may be, can be traced back to one and the same root. This wild stock is the Central Asiatic *Mus musculus wagneri* Eversmann, which is found all over the dry area of Central Asia from the Pacific, through northern China, Mongolia, Turkestan, and as far west as the Volga River. It has been possible definitely to prove that the development of the commensal out of the wild stock began in northern Persia, and that migration and the evolution of commensal types has taken place both in an eastern and western direction. Two different groups have been formed which are identical in most of the more typical commensal characters, although these have been developed independently of one another, only under the same conditions of habitat.

The eastern or Indian series inhabits Iran, India, Malaya, southern China and Australia; it has been carried into Oceania, and is known as far east as the Marquesas Islands. It is also the common type of house-mouse in East and South Africa, where it has been brought by shipping. The western, or European, series is found in Mesopotamia, northern Africa and western Europe. From there it has reached the Atlantic islands and has become a typical inhabitant of the American continent, being found both in the northern and southern hemispheres.

Let us now consider a few of the changes that can be observed in the evolution of the commensal mouse which distinguish it from the wild stock and which may be taken as typical commensal characters. I shall pick out (1) the concentration of the yellow pigment; (2) the pigment control; (3) the length of the tail; (4) the shape of the face and its relative length compared with that of the brain case.

In the Indian series four different kinds of yellow can be distinguished; they are buff, ochraceous, tawny and cinnamon. The palest shade occurs in the wild stock, ochraceous is found in the form *bactrianus,* which occurs in Iran and northwestern India. Tawny is typical of the Punjab commensal *tytleri* and of the Himalayan forms *homourus* and *urbanus;* the darkest stage, cinnamon, is found as an alternative in the same two forms. In short, the whole progress may be described as an intensification of the yellow tint which passes from buff to a shade which is rather reddish brown than yellow. All these stages represent mutations which make up an allelomorphic series of a variable factor which is designated as the concentration of yellow.

A similar series of mutations occurs in the factor that controls the distribution of pigment in general, and which is responsible for the demarcation between the color of the back and that of the belly. This series is well known to geneticists as the Agouti series. In the wild stock, *Mus musculus wagneri,* the belly is perfectly white, with

a sharp line of demarcation along the flanks, with the tail bicolored, and the hands and feet white and sharply set off. In the Punjab *tytleri* we find a buffy belly and buffy feet, with the line of demarcation slightly obliterated both along the flanks and on the tail. This type of coloration is also characteristic of the south European *Mus musculus brevirostris*. The next step in the series represents an animal with a gray belly and drab or sepia feet, without any line of demarcation along the flanks, and with a uniformly colored tail. This stage is represented by some of the Indian commensals, like *M. m. urbanus* and *M. m. manei*, and by the typical European *M. m. musculus*. The last stage of this series, all black, is not known anywhere in the mouse as a type forming whole populations, but it must be mentioned here because it occurs in the house-rat and the African Negro rat, and because we shall have to deal with it in connection with domestication.

As regards the length of the tail the wild stock has it much shorter or at most just as long as the head and body. But with advancing commensalism the tail may, and does, increase in length. True commensals may have the tail just slightly shorter than the body, but as a rule have it much longer. The genetic analysis of this factor is only in the beginning; but it appears that stages in tail length can be traced, and that they make up a series of alleles corresponding to the one studied by Madame Dobrovolskaia-Zavadskaia (1934) and in Professor Dunn's Laboratory in New York (Chesley and Dunn, 1935, 1936).

The changes of the skull that take place in the evolution of the house-mouse have been described by Gates (1926) in connection with his researches on the Japanese waltzing type. But they hold good for all commensals and serve to distinguish them from all the wild stocks. The chief character exhibited by the commensal is the shape of the facial portion, which is shorter than in the wild animal, with the rostrum more slender, compressed laterally and from above. How far these changes are inheritable, and in which way, nobody knows for certain, as the prob-

lem has not yet been touched by the genetic experiment. But they clearly represent a juvenile condition and may be due to some change in the endocrinal balance, which is controlled by unknown genetic factors.

It should be pointed out here that these mutations are not restricted to the mouse, but that they appear again in all the other commensal species.

VI

If we examine more closely these various series of mutations one fact becomes evident. All the mutations which appear in commensals and develop in a series are recessive mutations. This is perfectly clear and well established in the Agouti series. But from Nachtsheim's experiences (1929) in the rabbit the same applies for the concentration factor for yellow where the dominant mutations are pale, and the recessive mutations are intense. As far as we know at present, long tail is recessive to short tail, and again we have the recessive stage in the commensal and the dominant end of the series in the wild lot.

The question will be asked, are these recessive mutations produced by commensal conditions of life and why do they not appear in the wild stock. The reply is: they do appear in the wild stock, but being less resistant to wild conditions, they are either discarded because they have a reduced selective value or they look for shelter and become commensal. With increasing recessiveness commensalism becomes more strongly pronounced.

On the other hand, it is a fact that dominant mutations occur in the commensal as they do in the wild stock. Some of them occur in both[2] and do not seem to have any particular selective value under the conditions given. But from what we can ascertain from the actual commensal populations, it would appear that those alleles which are dominant over the local commensal type are not retained under commensal conditions. They either have a nega-

[2] One of these is the mutation "sharp dorsal zone," *i.e.*, a dark back sharply defined along the sides of the body; this corresponds to the "Verdunkelungsfaktor" of German authors.

tive selective value and disappear, or they run wild and become feral. Wheresoever we have two types of the same allelomorphic series in one area, the stage representing the recessive allele remains indoors, while the dominant type is found in the open.

VII

If recessive mutation proceeds, a stage will soon be reached where the disestablishment of the gene destroys all chance of surviving. Indeed, the lower stages of the albino series are entirely unable to exist even under commensal conditions. They have a reduced ability to produce immunity against infections, and exhibit all sorts of physiological disabilities.[3]

But quite a few of the more recessive mutations are able to exist in captivity or in a state of domestication. Domestication is but an exaggerated condition of what we find in the commensal stage, and in fact primitive commensals are not really different in their make-up from the commensal. They show, for instance, the same increase in yellow pigment, the same deficiency in pigment control, and the same type of juvenile skull which we have traced in the commensal mouse.

But in addition to that and increasing with more highly developed domestic conditions, we meet with more advanced stages of recessive mutation, such as the development of deep chestnut and brown out of the paler tints of the yellow series in the horse, cow, pig, but also whites and piebalds which in the wild stage, or even as commensals, would be impossible, except under very special conditions. This state of things is possible on account of the high degree of protection given by man to the domestic animal,

[3] See also Kleinschmidt (1936). The objection may be made that recessive mutations, even of the albino series, are able to survive in a wild state. Acromelanism, corresponding to Himalaya white, is not uncommon in birds, as for instance the tyrannid *Xolmis irupero* Vieillot or the fruit-pigeon *Ducula bicolor* Scopoli. But it appears that in these cases the recessive mutation fits into a balanced set of genes, whereas in the commensals under consideration the genetic balance has been upset under conditions of reduced control by natural selection.

but also to the process of artificial selection which preserves the dominant mutations, and thus counterbalances the evil effects of recessiveness in certain genes. Without this artificial process domestication would lead to degeneration and extinction. The partnership between man and the domestic animal, therefore, is an unequal one, the benefit, in a biological sense, being entirely on the side of the domestic animal.

VIII

This account would not be complete without a consideration of man himself, as he is the other partner of the commensal animal, and as he lives under the same general external conditions of environment. Has he the same tendency to produce recessive mutation? It would appear that in more than one character he follows the road of the commensal. Compared with primitive man, modern human types have a shortened face. Moreover, they have a tendency to become lighter and to lose the black pigment. From the limited amount of genetic data that we have from man, but more so from the data we have from Primates,[4] it can be taken as a well-supported theory that this development proceeds in a recessive direction. But even more than in the domestic animal this weakening of his constitution may be regarded as balanced, and overbalanced, by the protection man is enabled to provide for himself through his own intellect.

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SHORTER ARTICLES AND DISCUSSION

A REINVESTIGATION OF THE SO-CALLED SEX CHROMOSOMES IN *MELANDRIUM* (*LYCHNIS*) *ALBUM*

Melandrium (*Lychnis*) *album* has become one of the classic examples of sex determination dependent upon the behavior of a pair of unequal chromosomes during the reduction division. A reinvestigation of this angiosperm was undertaken to compare the results obtained from studies on the genus *Rumex*. In the dioecious members of the latter group various sex-determining mechanisms have been described among the chromosomes the nature of which involves an association of three bodies which segregate to the daughter nuclei of the pollen mother cells, adding two chromosomes to the one complement and one to the other. The behavior of this mechanism as described by numerous authors was found to be highly irregular. Many associated phenomena of the reduction divisions suggested a hybrid origin for these unisexual species. The same peculiarities ascribed to the sex chromosomes in the unisexual species were also found in the natural hybrids of some of the hermaphroditic species. Consequently, the author could hardly credit the peculiar history of certain chromosomes in the dioecious species as having a primary influence on the sex of any resulting generation.

With this in mind, the behavior of the so-called sex chromosomes in *Melandrium album* proves interesting. As in the case of *Rumex*, a superficial study might persuade one that the apparatus as described is without reproach. In this instance the sex chromosomes are supposed to form an unequal pair in the male during meiosis. So they do; but not always. The exceptions, most often found during the early anaphase, distinctly show that what might often appear to be an unequal pair is basically an equal pair, each member of the pair being in turn made up of two distinct pieces joined end to end. As in *Rumex*, where the supposedly tripartite chromosome revealed a tetrapartite nature, this supposedly unequal pair in *M. album* upon close study shows itself to be an association of two pairs of chromosomes. What makes them look like an unequal pair is due to a difference in the attachment and form subsequently taken by the halves of the

association. Often the end piece on one side is bent under the more equatorial piece so that a shortening seems to have occurred. It also appears that during the process of separation one of the equatorial members procures some of the chromatin from the other equatorial piece. Of course this adds to the inequality. During the second meiotic division the polar view of the metaphase plate often reveals that the so-called sex chromosome varies in form, depending presumably upon how much chromatin it either lost or gained in the first meiotic division.

A glance at the mature pollen sacs suggests that *M. album* too is of hybrid origin in that a great deal of imperfect pollen and occasional microcytes are present. The degree of imperfection varies with the anther sac; some being almost devoid of any perfect grains, while others possess largely perfect pollen.

In the author's opinion the validity of sex chromosomes in *M. album* is to be treated with skepticism because of two principal arguments. In the first place, the apparatus, when closely studied with improved technique, displays similarities to certain conditions in known hybrids and thus seems more closely associated with abnormalities induced by previous hybridization than with the direct determination of sex. It must also be pointed out that as one reviews the list of Angiosperms for which sex chromosomes have been claimed one is impressed with the fact that these very species belong to groups some of whose members are hermaphroditic and that they usually belong to fairly large genera in which natural hybridization is a common occurrence. Though research on the species in question has not been completed, there seems to be adequate reason to doubt the existence of a clear case for the presence of sex chromosomes in *M. album*.

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THE LONGEVITY OF INSECTS DURING COMPLETE INANITION

MY attention has been called to the fact that, in a recent paper (1938), I have set at odds, as it were, two prominent students of insect bionomics. The passage under concern (p. 41)ran thus: ". . . the mature larvae and adults of the North American grasshopper, *Chortophaga viridifasciata*, endure [approximately] the same length of time (5.5 to 6.6 days at all relative

humidities, including 0 per cent. R. H. Ludwig (1935) therefore suggested that the cause of death is starvation rather than desiccation. One would, however, suspect that the death of these grasshoppers was caused by a factor of which Ludwig was not aware, since Bodine (1921) had found that these grasshoppers "will live well beyond a hundred days without the intake of solids or water." Bodine, however, had stated "hours" and not "days." The results of the above investigators are thus in accord.

There exists a rather prevalent notion that insects are capable of marked resistance to complete starvation. This die-hard bias was undoubtedly the factor which caused me unintentionally to substitute "days" for "hours" when abstracting Bodine's paper. The following table, containing data collected at random, shows that most adult *active* insects are *in*capable of surviving more than a few to several days during complete starvation at ordinary temperatures and atmospheric conditions. Thus the active domestic fly (Glaser, 1923) can live for approximately 1.5 days without any food and water but will live about a month when normally nourished. A similar relationship holds for *Drosophila.*

There are, however, notable "exceptions." Thus, 8-mm larvae of the beetle, *T. tarsale,* can endure inanition for at least 6 years (see table). At the end of that time the larvae had become reduced to the size at hatching. Dufour (1933) kept an adult *Cimex lectularius* (bed-bug) in a closed container without food and water for a whole year (April, 1826–April, 1827), at the end of which time it was still vigorous. Kemper (1930) has recently made numerous extensive studies on the longevity of this bed-bug under various conditions and found a mean of 140 days for the longevity of the adults during inanition; and this only after the animals had completed a repast of mammalian blood (*cf.* table). The wife of a Belgian official in the Congo told me, several years ago, of a certain poisonous beetle in that country which is believed to be capable of enduring 8 years in complete starvation. I would like to learn, from scientific sources, more about this beetle.

The larvae of insects which undergo an extensive metamorphosis, *i.e.,* of insects which have a relatively pronounced fat-body, can endure starvation for a longer period than the adults of the same species. This is probably because of the large nutritive depôt and higher water percentage of the larvae. The main source of energy, during the starvation of insects, is fat

TABLE 1

Species (adults unless otherwise specified)	Approx. mean time (in days) for death during complete abstinence from food and water	Temp.° C.	Investigators
ORTHOPTERA			
Melanoplus femur-rubrum	2	22–25	J. H. Bodine (1907)
M. differentialis	3	"	"
Chortophaga viridifasciata (hibernating larvae)	7	23	"
C. viridifasciata (larvae and adults)	6	25	D. Ludwig (1935)
Periplaneta orientalis	20–60	Room	E. W. Sanford (1918)
NEUROPTERA			
Hemerobius sp.	2	"	H. J. Lucas (1826)
Perla bicaudata	2.5	"	"
HEMIPTERA			
Trialeurodes vaporarium	42	22	H. Weber (1931)
Cimex lectularius (bed-bug)			
Stage I larva (from completion of meal)	70	22	H. Kemper (1930)
Stage I larva (sobered)	35	"	"
Stages II and III (from completion of meal)	112	"	"
Stage V (from completion of meal)	133	"	"
Young males and females (from completion of meal)	140	"	"
C. lectularius (adult)	365 (at least)	Room	L. Dufour (1833)
ODONATA			
Libella cancellata	3	"	B. Slowtzoff (1905)
COLEOPTERA			
Chrysomela populi	6	"	F. L. A. W. Sorg
Dermestes lardarius }	13	"	"
Cerambyx fulginator }			
Lampyris noctiluca	36	"	" *cf.* Lucas (1826)
Coccinella 14-guttata	2	"	Fingerhuth, unpublished (*cf.* Lucas 1826)
Calandra granaria	3	"	Fingerhuth, unpublished (*cf.* Lucas 1826)
Curculio scrophularia	5	"	Fingerhuth, unpublished (*cf.* Lucas 1826)
Melolontha horticula	6	"	Fingerhuth, unpublished (*cf.* Lucas 1826)
M. vulgaris	21	"	Slowtzoff (1904)
Geotrupes stercoralis	8	"	Slowtzoff (1909)
Geotrupes sp.	14	"	Fingerhuth
Lucanus cervus	27	"	"
Cetonia aurata	30	"	"
Tenebrio molitor (larva)	ca. 240	27	H. Sing-Pruthi (1925)
T. molitor	30	Room	G. Teissier (1928)
Trogoderma tarsale (museum pest)			
(1-mm. larva)	ca. 240	"	J. E. Wodsedalek (1917, 1921)
(8-mm. larva)	2,190 (at least)		
LEPIDOPTERA			
Bombyx cerura (*does not* take food normally)	15	Room (June)	Lucas
B. mori (does not take food normally)	3	" "	"
B. mori (larva)	8	" "	V. L. Kellogg and R. G. Bell (1904)
Deilephila euphorbiae	12	" "	K. Farkas (1903)
Sphinx moth	12	" "	J. Heller (1926)

TABLE 1—(*Continued*)

Species (adults unless otherwise specified)	Approx. mean time (in days) for death during complete abstinence from food and water	Temp.° C.	Investigators
HYMENOPTERA			
Formica fusca	2	" (May)	Lucas
Apis terrestris	3	" "	"
A. mellifica	5	" (June)	"
Vespa vulgaris	8	" "	"
Bombus terrestris	1.5	" "	Slowtzoff (1905a)
DIPTERA			
Ophyra cadaverina	6	" "	F. Tangl (1909)
Musca domestica	1.3 (without water)	" "	S. I. Vinokuroff *cf.* Pearl and Parker (1924)
	1.8 (with water)		
M. domestica	2		R. W. Glaser (1923)
Drosophila melanogaster (wild and vestigial-winged)	2 (both sexes; ♀♀ somewhat longer than ♂♂)	25	R. Pearl and S. L. Parker (1924), and W. M. Barrows (1907)
D. ampelophila	2 (same remark as above)	25	F. E. Lutz (1915)

(Slowtzoff, 1904, 1905, 1909, 1905a, Tangl, 1909, Kopeć, 1924, Heller, 1926), and Slowtzoff believed that in most such cases death ensues when the fatty stores have been largely consumed. At the time of death the percentage of dry substance lost is approximately constant. Teissier (1928) found this to be true regardless of the temperature (10°–37° C.).

Next to fat, under ordinary atmospheric conditions, water is the substance lost to a most marked extent (expressed in per cent. of the initial quantity of water). Since the bumble-bee and dragon-fly die after losing only a relatively small fraction of their fats, Slowtzoff believes that water-loss is a prominent factor in the short duration of these insects during starvation, even though they do not lose more water than several other species which live longer. In accordance, a great deal of evidence can be presented to show that insects vary considerably in their tolerance to water-loss. In the instance of *C. viridifasciata* (*vide supra*) water-loss is apparently not the limiting factor. In fact, the water *percentage* of this animal rises when starved at an R. H. of 96 per cent. (Ludwig, 1935); but this does not, however, imply that a loss in the total water *content* does not occur. In most cases (including other grasshoppers, studied by Bodine, 1921) it is clear that water-loss is, other things being equal, a limiting factor, since longevity during starvation is considerably prolonged if water is provided (see, for instance, Bodine, 1921, Vinokuroff, see table).

On the whole, it appears that the duration of life during complete starvation is, to a certain extent, a function of the normal life span and this again is, to a certain degree, a function of the metabolic rate. Such a generalization is not amenable to rigid application on its sole merits. Thus, while the normal life span of the wild type *Drosophila melanogaster* (Pearl and Parker, 1924) is about three times that of the vestigial-winged type, the duration of both types during inanition is equal. I have plotted data collected at random in an attempt to exhibit a supposed relationship between the normal metabolic rate (oxygen consumption rate) at a given temperature (20°–25° C.) and the duration of life during inanition of species exemplifying approximately the entire animal kingdom and find no general relationship between such; this is true even when the plottings are restricted to a single class—the insects.

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FURTHER STUDIES ON INCUBATION OF TURTLE (*MALACLEMYS CENTRATA* LAT.) EGGS[1]

In earlier reports (Cunningham and Hurwitz, 1936, and Cunningham and Huene, 1938) it was shown that the increase in weight of various reptile eggs during incubation could be accounted for, at least for the most part, by the absorption of water.

The studies did not positively exclude the possibility that mineral salts were also taken up by the egg. An examination of the data of Karashima (1929) indicates that both mineral salts and carbohydrates increase in the first fifteen-day period of incubation in the albumen and yolk of the sea turtle, *Thalassochelys corticata*. The source of this increase was not specified, although it may be assumed to be the shell. The present studies consider the whole egg as a unit and attempt to discover whether minerals from the environment enter the egg.

[1] These experiments were carried out at the U. S. Fisheries Laboratory at Beaufort, N. C., and the authors express appreciation to the bureau for the use of its facilities, and to Dr. H. F. Prytherch, director, and Mr. Charles Hatsel, foreman, for their cooperation.

It is unfortunate that the complete story can not be followed in each egg and that a somewhat statistical method has to be followed in order to ascertain whether or not elements other than water are added from the environment during incubation. Such procedure involves the use of a reasonable number of eggs at each of the selected ages. In these experiments the number was limited by the time available for the analyses. The eggs were studied individually, since by this method significant variations might become evident.

Newly laid eggs were taken from the nest before it was covered. These were weighed and immediately desiccated and reweighed to a constant figure. The dried mass was then ashed and weighed. Other eggs were incubated for 33 days under normal conditions and a similar procedure followed. Still others were incubated for 60 days and similar data collected.

The composite data for each group are shown in Table I. The data show a very slight increase in dry weight in the 33-day group over the fresh eggs. This would appear, however, to be well within experimental error. The increase in ash weight, however, is greater, but when taken into consideration with the slight rise in organic materials it is probably again within the range of experimental error.

TABLE I

WEIGHTS OF ASH AND ORGANIC MATERIAL AT VARIOUS STAGES OF DEVELOPMENT

No. eggs	Days incubated	Average wet wt.	Max. Min.	Average dry wt.	Max. Min.	Average org.* wt.	Max. Min.	Average ash wt.	Max. Min.
20	0	10.581	11.668 7.283	3.117	3.445 1.829	2.764	3.072 1.574	.3531	.4341 .2478
20	33	12.441	14.826 10.701	3.155	3.672 2.769	2.784	3.356 2.529	.3714	.4313 .3120
25	60	13.326	16.585 11.273	2.800	3.645 2.005	2.429	3.090 1.727	.3712	.4160 .2597

* Organic material.

The variations in dry weight, ash content and organic material between eggs in a single group are far greater than the differences in averages of the different groups. While there is much overlapping from one group to the other the maximum ash in each

group shows a steady but comparatively slight regression during incubation.

Taken as a whole, it appears as if the mineral salts (ash) are reasonably constant during incubation. Further evidence for this conclusion, secured by a different procedure, will be presented later. The slight rise in organic material, when there should have been an appreciable loss at 33 days, can not be so easily explained, but the considerable drop before the 60th day indicates it may be an error in technique; it might be accounted for either by proportionately thicker shells or larger yolks in this particular group of eggs.

The problem of absorption of minerals was also approached from another angle. Eggs were placed upon a rack in a humidity chamber. A piece of cheese-cloth, one end of which dipped into distilled water, was placed under the eggs and folded back so as to cover the tops of the eggs. A glass plate, slightly raised to allow admission of air without too rapid evaporation of water, was placed over the top of the chamber. The distilled water was changed from time to time to prevent stagnation. A control, using tap

TABLE II

COMPARISON OF NEWLY HATCHED TURTLES FROM EGGS SUPPLIED WITH DISTILLED WATER ONLY WITH THOSE INCUBATED IN NATURE

	Number embryos	Average wet wt.	Max. Min.	Average dry wt.	Max. Min.	Average organ. wt.	Max. Min.	Average ash wt.	Max. Min.
Normal	10	7.860	9.8141	2.138	2.9056	1.915	2.636	.223	.2693
			7.2409		1.9710		1.754		.2054
Experimental	5	7.517	7.8594	2.055	2.2412	1.830	1.976	.225	.2485
			7.2613		1.9382		1.721		.2107

water (artesian well), was also set up. The jars were exposed to ordinary room temperature. In both cases the development proceeded to hatching, and gross examination revealed no anomalies of development, either in form or time required for hatching as compared to the normal in nature. The newly hatched embryos, supplied during incubation with distilled water, when analyzed and compared with embryos hatched under normal conditions, showed no significant differences. The data are given in Table II.

It is quite evident that all the mineral salts necessary for development are contained in the eggs; in fact, there is an excess, which may be partly accounted for by the minerals remaining in the shell at the time of hatching.

These experiments indicate that when the egg of the diamond back terrapin is taken as a whole it contains (with the exception of water) all the ingredients, in sufficient quantity, to insure the proper development of the embryo.

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